Manganese Dosimetry: Species Differences and Implications for Neurotoxicity

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Aschner, M., Erikson, K.M., and Dorman, D.C. (2005) Manganese Dosimetry: Species Differences and Implications for Neurotoxicity. Critical Reviews in Toxicology. 35(1):1-32.

Abstract:
Manganese (Mn) is an essential mineral that is found at low levels in food, water, and the air. Under certain high-dose exposure conditions, elevations in tissue manganese levels can occur. Excessive manganese accumulation can result in adverse neurological, reproductive, and respiratory effects in both laboratory animals and humans. In humans, manganese-induced neurotoxicity (manganism) is the overriding concern since affected individuals develop a motor dysfunction syndrome that is recognized as a form of parkinsonism. This review primarily focuses on the essentiality and toxicity of manganese and considers contemporary studies evaluating manganese dosimetry and its transport across the blood—brain barrier, and its distribution within the central nervous system (CNS). These studies have dramatically improved our understanding of the health risks posed by manganese by determining exposure conditions that lead to increased concentrations of this metal within the CNS and other target organs. Most individuals are exposed to manganese by the oral and inhalation routes of exposure; however, parenteral injection and other routes of exposure are important. Interactions between manganese and iron and other divalent elements occur and impact the toxicokinetics of manganese, especially following oral exposure. The oxidation state and solubility of manganese also influence the absorption, distribution, metabolism, and elimination of manganese. Manganese disposition is influenced by the route of exposure. Rodent inhalation studies have shown that manganese deposited within the nose can undergo direct transport to the brain along the olfactory nerve. Species differences in manganese toxicokinetics and response are recognized with nonhuman primates replicating CNS effects observed in humans while rodents do not. Potentially susceptible populations, such as fetuses, neonates, individuals with compromised hepatic function, individuals with suboptimal manganese or iron intake, and those with other medical states (e.g., pre-parkinsonian state, aging), may have altered manganese metabolism and could be at greater risk for manganese toxicity.
Keywords: Biomarkers, Brain, Manganese, Metabolism, Neurotoxicity, Pharmacokinetics

Article:
I NTRODUCTION
As with other essential minerals, there is a dichotomy to the response of the body to manganese. Adverse health effects can occur when body stores of manganese are either too low (deficiency) or too high (toxicity). Manganese deficiency has been recognized in experimental animals and is associated with impaired growth, skeletal defects, reduced reproductive function, and abnormal glucose tolerance, as well as altered lipid and carbohydrate metabolism.[1,2] Because of its widespread presence in human diets, manganese deficiency is generally not recognized in humans. Under certain high-dose exposure conditions manganese may accumulate within the human body, and it can induce adverse neurological, reproductive, and respiratory effects. Manganese neurotoxicity is especially pernicious and in chronic states is characterized by damage to dopaminergic neurons within brain regions that control muscle movement.[3-5]

For most individuals, the primary source of manganese is the diet. Only a small percentage of the ingested manganese is absorbed and even less is ultimately delivered to the brain. Brain delivery of manganese is regulated by a number of physiological systems that includes the normal function of the gastrointestinal tract, hepatobiliary system, transferrin and other blood trans-port proteins, and the blood—brain barrier. A hallmark feature of manganese neurotoxicity is the development of increased brain manganese concentrations.

Abnormally high brain manganese delivery can occur when these regulatory systems are partially bypassed as in the case of inhalation[3,6,7] or when these control systems are overwhelmed due to the ingestion of extremely high levels of manganese in drinking water.[8,9] Enhanced brain manganese delivery may also occur when hepatobiliary or other disease states compromise normal manganese homeostasis.

There is increased interest within the scientific community on the neurotoxicity of manganese. Manganese is ubiquitous in the atmosphere, and it is associated with numerous industrial processes.[10] Other factors that have fostered this attention include the increasing worldwide use of methylcyclopentadienyl manganese tricarbonyl (MMT) as a gasoline fuel additive and an enhanced awareness that manganese may play a role in hepatic encephalopathy and other neurologic diseases. Another recent development is the application of modern brain imaging techniques to the evaluation of brain manganese deposition. These imaging studies are likely to yield important new insights into manganese-induced neurotoxicity. Manganese exposure is likewise the interest of numerous governmental regulatory agencies. Manganese is listed as an air toxic by the U.S. Environmental Protection Agency (U.S. EPA). The U.S. EPA (see http://www.epa.gov/iris/subst/0373.htm) has established an inhalation reference concentration for respirable manganese (0.05 μg Mn/m^3). Environmental monitoring studies have shown that human exposures are often below this leve1.[11]

This review focuses on the dosimetry of manganese and provides information about exposure conditions that lead to increased concentrations of the metal within the central nervous system (CNS) and other target sites. This review is not meant as an exhaustive listing of the manganese literature; rather, it is intended to provide a useful synopsis of contemporary studies from which the reader may progress to other research citations as desired. Specific emphasis is directed at recent published literature on manganese toxicokinetics. Issues specifically addressed in this review include the following: (a) the essentiality and toxicity of manganese; (b) the absorption, distribution, metabolism, and elimination of manganese in the general population and potentially susceptible subpopulations; and (c) potential biomarkers of manganese exposure. Neurological effects induced by manganese are a special focus throughout this article.

MANGANESE ESSENTIALITY AND TOXICITY

Manganese Essentiality

Manganese is an essential trace metal that is found in all tissues and is required for normal amino acid, lipid, protein, and carbohydrate metabolism. Manganese-dependent enzyme families include oxidoreductases, transferases, hydrolases, lyases, isomerases,. and ligases. Manganese metalloenzymes include arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and manganese superoxide dismutase. Manganese is involved in the function of numerous organ systems, and it is needed for normal immune function, regulation of blood sugars and cellular energy, reproduction, digestion, bone growth, and it aids in defense mechanisms against free radicals. Manganese in concert with vitamin K supports the clotting of the blood.

No formal Recommended Dietary Allowance (RDA) for manganese has been established, but the U.S. National Research Council has established an estimated safe and adequate dietary intake (ESADDI) of 2-5 mg/day for adults.[13] Factors that influence the daily manganese requirement have been recently reviewed.[14] The adequate intake for manganese for adult men and women is 2.3 and 1.8 mg/day, respectively.[14] Finley et al.[15] reported that men absorbed significantly less manganese than women from the gastrointestinal tract. It has been postulated that this decrease in gastrointestinal manganese absorption is related to iron status and the lower serum ferritin concentrations found in men.[14,15] Lactation and gestation are thought to increase the *manganese* requirement.[14] Developmental life stage can also influence dietary manganese requirements. Adequate intakes for newborn (<6 months of age) infants are approximately 3 μg/day, while intakes increase to 600 μg/day by 7 to 12 months of age.[14] Children 1-3 and 4-8 years of age have adequate daily manganese intakes of approximately 1.2 and 1.5 mg/day.

Manganese Deficiency

Manganese deficiency can result in a host of adverse health effects, including impaired growth, poor bone formation and skeletal defects, reduced fertility and birth defects, abnormal glucose tolerance, and altered lipid

and carbohydrate metabolism.[1,2] Al-though manganese deficiency has been observed in animals, to our knowledge, frank manganese deficiency has not been clinically recognized in humans. Some clinical signs and symptoms have been observed in human subjects placed on manganese deficient diets. Young men placed on manganese-depleted diet developed an erythematous rash on their torsos.[16] Penland and Johnson reported that young women consuming a diet containing only 1 mg Mn/day developed altered mood and increased pain during the premenstrual phase of their estrous cycle.[17]

Manganese intake by many individuals remains below the recommended dietary reference intake level of 1.8 to 2.3 mg/ day.[18,19] Even when dietary levels of manganese are adequate, high dietary levels of fiber, phytate, ascorbic acid, iron, phosphorus, and calcium can limit the oral bioavailability and retention of manganese.[13,14,20-23] Suboptimal manganese status may also occur in humans with epilepsy, osteoporosis, or exocrine pancreatic insufficiency, individuals undergoing chronic hemodialysis, and in children with Perthes's disease or phenylketonuria.[1,24]

Manganese Toxicity

Manganese toxicity in humans is a well-recognized occupational hazard for people who inhale manganese dust. Inhalation of particulate manganese compounds (e.g., manganese dioxide [$Mn0_2$] or manganese tetroxide [Mn_30_4]) leads to an inflammatory response in the lungs of animals and humans. Respiratory clinical signs and symptoms include cough, bronchitis, pneumonitis, and impaired pulmonary function.[25] These effects may reflect an indirect response to inhaled particulate matter or may be associated with direct pulmonary toxicity induced by manganese.[6] Impotence and loss of libido are another set of common symptoms observed in male workers with exposure to high concentrations of manganese.[6] Although these effects are important, manganese-induced neurotoxicity (manganism) is of greatest concern and is considered to be one of the most sensitive toxicological endpoints in people and animals.

Air manganese concentrations reported to be associated with signs consistent with manganese neurotoxicity range from 0.027 to 1 mg Mn/m.[3,7,26-32] These values represent estimates of actual exposure levels and provide limited dose-response information. Frank manganese neurotoxicity most commonly occurs in workers that have been chronically exposed to aerosols or dusts that contain extremely high levels (>1-5 mg Mn/m3) of manganese.[3,6,7] Manganese-induced neurotoxicity may also occur following ingestion. Kawamura and coworkers[8] and Kondakis et al.[9] documented outbreaks of manganese toxicity in Japan and Greece, respectively, due to the consumption of water from wells contaminated with extremely high levels of manganese (1.8 to 14 mg Mn/L). More recently, Woolf et al. (2002)[33] reported that a 10-year-old boy with abnormal verbal and visual memory function had elevated serum (0.90 µg/dl vs. normal value of <0.265 µg/dl), whole blood, urine, and hair manganese concentrations following chronic ingestion of well water containing modestly elevated levels (~1.2 ppm) of manganese. Along with the other reports already noted, this case suggests that children might be more sensitive than adults to excessive oral manganese exposures.

Manganism is associated with elevated brain levels of manganese, primarily in those areas known to contain high concentrations of nonheme iron, especially the caudate-putamen, globus pallidus, substantia nigra, and subthalamic nuclei. Manganism is initially characterized by a psychiatric disorder *(locura manganica)* that resembles schizophrenia. Symptoms include compulsive and violent behavior, emotional instability, and hallucinations. Other early manifestations of manganese neurotoxicity include fatigue, headache, muscle cramps, loss of appetite, apathy, insomnia, and diminished libido.[3] As exposure continues and the disease progresses, patients may develop prolonged muscle contractions (dystonia), decreased muscle movement (hypokinesia), rigidity, and muscle tremors.[3] These signs are associated with damage to dopaminergic neurons within brain structures that control muscle movement.

Individuals with manganism resemble patients with Parkinson's disease; however, these syndromes can be distinguished clinically.[3-5] Similarities between Parkinson's disease and manganism include the presence of generalized bradykinesia and widespread rigidity. Dissimilarities between Parkinson's disease and manganism were also recognized, notably the following in manganism: (a) a less frequent resting tremor, (b) more frequent

dystonia, (c) a particular propensity to fall backward, (d) failure to achieve a sustained therapeutic response to levodopa, and (e) failure to detect a reduction in fluorodopa uptake by positron emission tomography (PET).[3,5] Based on these differences, it has been proposed that manganese intoxication is associated with preservation of the nigrostriatal dopaminergic pathway, and that chronic manganese intoxication causes parkinsonism-like effects by damaging output pathways downstream of the nigrostriatal dopaminergic pathway.[3,5,34]

Adverse effects of manganese on the developing nervous system are also a concern. There have been sporadic cases in which children with excessive exposure to manganese have developed signs of neurotoxicity.[33,35,36] When compared to adults, neonatal rodents are at an increased risk for manganese-induced neurotoxicity due to their ability to achieve higher brain manganese levels and altered brain dopamine concentrations following similar oral exposures.[37-40] Known pharmacokinetic processes that may contribute to the increased susceptibility of neonatal animals include increased manganese absorption from the gastrointestinal tract, an incompletely formed blood—brain barrier, and reduced biliary manganese excretion in preweanling animals.[41-43] The relative higher net increase in brain manganese concentrations in the neonates (vs. adults) should not necessarily be construed as evidence for heightened neurotoxicity in developing animals, given the known requirement for manganese for optimal CNS development in the neonate. Manganese concentration in the brain of developing rats is highest of all age groups, suggesting that manganese is required in a high amount during infancy, and that a sufficient manganese supply is critical for normal brain development.[44]

A recent study by Dorman et al. (2000)[37] assessed the relative sensitivity of neonatal and adult CD rats to manganese-induced neurotoxicity. Identical oral manganese chloride ($MnCl_2$) doses (0, 25, or 50 mg/kg body weight/day) were administered to neonatal rats throughout lactation (postnatal day [PND] I though 21) and to adult male rats for 21 consecutive days. Increased striatal, cerebellar manganese concentrations were observed in adult rats from the high-dose group only. In contrast, increased striatal, hippocampal, hindbrain and cortical manganese concentrations were observed in all manganese-exposed neonates on PND 21. These results suggest that in the rat, manganese is more efficiently transported into the CNS of neonates compared to adults receiving equal oral doses of manganese.

A substantial literature base exists regarding significant species differences in the neurotoxicity of manganese. Ex-posed monkeys retain manganese in their basal ganglia; they develop reduced concentrations of dopamine and certain dopamine metabolites (3,4-dihydroxyphenylacetic acid) in their striatum and pallidus, have decreased dopamine binding, and develop loss of dopaminergic neurons analogous to manganese-poisoned humans.[5,45-48] However, similar regional brain manganese distribution, neurochemical, and neuropathological responses are not consistently observed in manganese-exposed rodents.[37,38,40,49-51] Neither do rodents develop behavioral syndromes comparable to that seen in manganese-poisoned humans and monkeys.[52] When considered together, these findings suggest that species differences confound the extrapolation of neurotoxicity results obtained for manganese in rodents to humans, possibly as a result of differential distribution of manganese in the CNS of rodents versus primates or due to intrinsic differences in tissue sensitivity in these various animal species.

SOURCES OF MANGANESE

Diet and Water

The most important source of manganese for the general population is diet, with most daily intakes falling below 5 mg Mn/kg. Adult dietary intake of manganese has been estimated to range from 0.9 to 10 mg manganese/day.[6,18] Manganese levels in excess of 30 mg/kg food can be found in grain, rice, and nuts. A cup of tea may contain as much as 0.4 to 1.3 mg manganese.[6] Another important source of manganese intake is the consumption of manganese-containing dietary supplements, where each tablet can contain 5-20 mg of manganese.[14] Water concentrations of manganese typically range from 1 to 100 µg/L with most values below 10 µg/L.[53] Based on the Total Diet Study, in adult males, grains, beverages (tea), and vegetables provide approximately 33, 20, and 18% of dietary manganese, respectively.[54] The U.S. EPA has established an oral reference dose (RfD) for manganese (0.14 mg/kg-day; see http://www.epa.gov/iris/subst for additional information).

Milk and Infant Formulas

In general, milk is low in manganese; however, concentrations found in human and cow's milk can vary dramatically, with published values ranging from 3 to 120 and from 30 to 50 μg/L, respectively.[53,55-57] This wide range of manganese concentrations may reflect in part different dietary levels of manganese. Manganese content in human (and animal) milk also varies with the stage of lactation[56-58] (Table I). For example, Stastny and coworkers reported that mean (±SD) human milk manganese concentrations in the fourth week of lactation were 6.6 ± 4.7 μg/L, and these levels were significantly higher than those collected during the 12th week of lactation (3.5 ± 1.4 μg/L).[56] Manganese intake in formula-fed infants is much higher than that observed in the breast-milk fed counterparts (Table 1) since levels of manganese in infant formulas may be substantially higher than that found in human milk.[59] Nutritionists have recognized the risk posed by high manganese concentrations in infant formulas, and levels of manganese found in these formulas have changed dramatically over the last 20 years. Prior to 1983, Enfamil (Mead Johnson and Company) contained 1289 μg Mn/L, while manganese levels dropped to 105 μg/L shortly thereafter.[56] Despite these modifications, consumption of soy-based and other infant formulas remains a potential concern for human infants.[60]

TABLE I
Manganese concentration in human milk and select infant formulas

Investigator	Country	Analysis method	Study description (number of subjects)	Human milk (μg Mn/L)	Infant formula (μg Mn/L)
Krachler et al., 2000[59]	Austria	ICP-MS	Human milk (27)	Observed range: 1.8–22.3	Observed range: 32.8–55.0*
			Infant formula (4)[a]		
Casey et al., 1989[58]	United States	GFAAS	Human milk (22)	Observed range: 1.97–3.94	
Stastny et al., 1984[56]	United States	GFAAS	Human milk (116)	Observed range: 1.9–27.5	Observed range: 70–1289**
			Infant formula (7)[b]		
Al-Awadi and Srikumar, 2000[280]	Kuwait	GFAAS	Human milk (17)	Observed range: 3.8–6.0	

Note. Data are ranges of means for each parameter.

GFAAS, graphite furnace atomic absorption spectrophotometry; ICP-MS, inductively coupled plasma mass spectrophotometry.

[a]Represents four different formulas tested, with at least six samples of each.

[b]Represents seven different formulas tested; Similac (Ross Laboratories) had the lowest Mn, whereas Enfamil (Mead Johnson and Company) had the highest. *Note*. As of March 1983, Enfamil is formulated to contain 105 μg/L.

Airborne Manganese

Although inorganic manganese compounds are not volatile, they can exist in air as fumes, aerosols, or suspended particulate matter.[6] Atmospheric manganese derives from both anthropogenic and natural sources.[61] The wind erosion of dusts and soils and their subsequent reentrainment in the air contribute to airborne manganese. Industries associated with manganese emissions include ferroalloy production, iron and steel foundries, metal fumes from welding, battery production, and power plant and coke oven combustion emissions.[10] Most human exposures remain below the current inhalation reference concentration (RfC) (0.05 μg Mn/m^3) for inhalable manganese set by the U.S. EPA.[62-64] Average levels of manganese in ambient air are approximately 5 and 33 ng Mn/m^3 in nonurban and urban air, respectively,[6] although air concentrations may be higher near ferromanganese or silicomanganese industries. The daily intake of manganese from the ambient air is estimated to be <2 μg Mn/day in the general population.[64-65]

Manganese is also found in methylcyclopentadienyl manganese tricarbonyl (MMT), a fuel additive used in some unleaded gasolines. MMT is presently available in the United States, Canada, and other countries for use in fuel, replacing lead as a gasoline additive. Combustion of MMT by the automobile engine results in the formation and emission of a complex mixture of phosphate, sulfate, and oxide forms of manganese.[65-68] The use

of MMT in fuel has been the subject of much debate and review by regulatory agencies in Canada and the United States.[69-72]

Air manganese concentrations from Toronto and Montreal, cities in which MMT has been widely used for over 10 years, remain near or below the current inhalation reference concentration (RfC) (0.05 μg Mn/m^3) for inhalable manganese set by the U.S. EPA.11,62-64 For example, the average atmospheric manganese concentrations measured in a high traffic area in Montreal were 0.050 and 0.024 $\mu g/m^3$ for total and respirable fractions, respectively.[63,64] In an area of much lower traffic density, total and respirable fractions were 0.027 and 0.015 $\mu g/m^3$, respectively.[63] Personal exposures to manganese showed that the 99th percentile nonoccupational exposure to respirable manganese ($PM_{2.5}$) in Toronto was 0.0215 μg Mn/m^3[3,11,62]

Ambient manganese concentrations have also been measured in urban air where MMT is not used. For example, Pellizzari et al.[73] have completed a smaller scale air-monitoring study in Indianapolis, a city where MMT is not used in gasoline. The levels of airborne manganese in Indianapolis were generally slightly lower than in Toronto. The median and mean levels for personal $PM_{2.5}$ Mn exposures in Indianapolis were 0.0028 and 0.0075 $\mu g/m^3$, respectively, while the mean and median exposures for the nonoccupationally exposed group were 0.0026 and 0.0031 $\mu g/m^3$, respectively. Because there are many anthropogenic sources of environmental manganese, and the importance of these sources varies from city to city, it is not possible to ascribe all the differences in exposures between Toronto and Indianapolis to MMT. Moreover, it is unknown whether the difference in measured exposures in the two cities are statistically significant.

Parenteral Exposure

Individuals undergoing total parenteral nutrition (TPN) are at risk for manganese neurotoxicity. Parenteral nutrition solutions are sometimes formulated to include manganese.[74,75] Wilson et al.[76] found that manganese concentrations in TPN fluids ranged from 5.6 to 8.9 μg/L.

Few studies have addressed the kinetics of manganese in the body upon TPN administration.[77] Furthermore, no clear standard has been recommended for the daily dose of manganese, with the published literature indicating a broad, 200-fold range in the recommended daily manganese dose for adults, extending from a low dose of 0.18-0.91 μmol (0.01-0.05 mg) to a high dose of 40 μmol (2.2 mg).

Manganese intoxication associated with TPN solutions pro-viding 0.1 mg Mn/day have been reported.[46,78,79] As with other forms of manganese poisoning, these patients developed elevated serum manganese level, they had symmetrical high-intensity magnetic resonance imaging (MRI) lesions in the globus pallidus consistent with manganese accumulation at this site, and they developed characteristic psychiatric symptoms and clinical signs of manganese-induced neurotoxicity.77 Withdrawal from the TPN supplement significantly decreased manganese levels in both the blood and brain.[78,80]

Another potential source of manganese exposure is the inclusion of manganese in radiological contrast agent.[81-83] In each of these cases, parenteral exposure may pose a much higher risk for manganese toxicity since the bioavailability of manganese in parenteral fluids is much higher than that observed following oral exposure (100 vs. ~5%). Moreover, normal regulation mechanisms for manganese metabolism are largely bypassed, and there is a greater danger of toxicity. Infants and adults with hepatic insufficiency are at an even greater risk for developing increased serum or tissue manganese concentrations when kept on prolonged parenteral nutrition.74

Other Sources

Humans may also be exposed to organic manganese-containing pesticides, such as manganese ethylene-bis-dithiocarbamate (Maneb).[84] Another potential source of manganese exposure is the street drug "Bazooka," which is a cocaine-based street drug contaminated with manganese carbonate.[85]

MANGANESE ABSORPTION

Oral Absorption

Only a small fraction (1-5%) of ingested manganese is normally absorbed into the body.[86] Most estimates of net gastrointestinal absorption of manganese in humans have been based on studies evaluating the whole-body retention of radiolabeled manganese (^{54}Mn) several weeks after an oral exposure to a tracer dose of ^{54}Mn. Finley and coworkers15 estimated that net gastrointestinal absorption (mean ± SD) of manganese from a meal containing 1 mg manganese was 1.35 ± 0.51% and 3.55 ± 2.1 % for adult men and women, respectively. Davidsson et al.[87] found that mean (±SD) retention 10 days after ingestion of approximately 0.3 mg manganese was 5.0 ± 3.1% in young adult women. In contrast, oral tracer studies that evaluate the retention of manganese less than 24 h after manganese ingestion reveal a much higher initial retention of the ingested dose. Animal studies conducted with diets spiked with a tracer dose of ^{54}Mn confirm that much of the retained manganese is found in the liver and the intestinal tract. The majority of this manganese does not become available to systemic tissues.

The mechanism by which manganese is absorbed from the gastrointestinal tract is not completely understood. Some studies suggest that manganese is absorbed through an active transport mechanism.[88] Other investigators have shown that manganese absorption may occur via a nonsaturable process consistent with passive diffusion.[89] Under normal conditions, the manganese arriving at the liver in the portal circulation is protein bound. Within the plasma approximately 80% of manganese is bound to β-globulin and albumin, and a small fraction of trivalent (3+) manganese is bound to the iron-carrying protein transferrin.[90,91]

Manganese absorption from the gastrointestinal tract is extremely complex, and a number of factors can influence manganese absorption and retention. The concentration of manganese in the diet is known to influence the amount of manganese absorbed from the gastrointestinal tract and the amount of manganese eliminated in the bile. When dietary manganese levels are high, adaptive changes include reduced gastrointestinal absorption of manganese, enhanced manganese liver metabolism, and increased biliary and pancreatic excretion of manganese.[18,86,92-97] For example, the relative amount of manganese absorbed from the gastrointestinal tract decreased as the dietary manganese concentration increased from 4 to 2000 ppm.[98] Abrams et al. (1976)[98] also showed decreased manganese retention (expressed as the percent dose per gram tissue) in the liver, kidney, spleen, and other systemic tissues as the concentration of manganese in the diet increased.

Davidsson et al.[99] demonstrated that the fractional manganese absorption from human milk was significantly higher than that observed in adult volunteers given a soy-based diet (8.2 ± 2.9 vs. 0.7 ± 0.2%) that provided 132 μg manganese (vs. 7.2 μg Mn in the human-milk diet). Net manganese absorption from the diet is also influenced by the presence of other trace minerals, phytate, ascorbic acid, and other dietary constituents.[20] The potential for manganese—nutrient interactions in the gastrointestinal tract makes it difficult to predict the delivered dose of manganese arising from a given dietary exposure.

Manganese absorption may in part involve complex interactions with other minerals.[100] Lai et al. used a developmental rat model of chronic manganese toxicity to evaluate these nutrient interactions.[100] Lai and coworkers treated neonatal rats with a high concentration of manganese chloride (20 mg $MnC1_2$/ml drinking water) throughout development until adulthood.[100] Administration of manganese in drinking water was associated with increased levels of iron, copper, selenium, zinc, and calcium in various regions of the brain. Planells et al. demonstrated that enterocytes from Wistar rats maintained on a magnesium-deficient diet (129 mg Mg/kg food for 70 days) had higher manganese concentrations when compared with control animals fed a diet that met nutritional requirements for magnesium (480 mg Mg/kg food).[101] Davidsson et al. demonstrated an inverse relationship between manganese absorption and the concentration of calcium found in human milk.[20] Although these interactions are important, manganese interactions with iron have been the most extensively studied. Animal studies have demonstrated that iron deficiency enhances manganese absorption across the gastrointestinal tract, independent of body manganese stores.[102,103] An inverse association between body iron stores and manganese absorption has also been demonstrated in humans.[104] Competition between manganese and iron for intestinal absorption[105] likely occurs by way of divalent metal transporter 1, referred to as DMT-1

(also known as DCT- 1 or nramp-2).[106] Functionally, DMT-1 mediates the intestinal uptake of numerous divalent metal cations, and DMT-1 mRNA levels in the duodenum strongly increase in response to iron depletion. [107] Studies in Caco-2 cells, in an in vitro model of the gastrointestinal epithelium reveal that iron treatment decreases cellular uptake of both iron and manganese (as well as zinc), suggesting that these metals may utilize the same apical and basolateral transporters.[108]

Manganese absorption from the gastrointestinal tract is also influenced by age. Studies conducted in suckling rat pups show that manganese absorption is very high during the neonatal period.[109,110] Rat pups younger than 15 days of age given human milk labeled with ^{54}Mn retained approximately 80% of their oral exposure dose 6 h after ingestion. Older rats (> 16 days of age) had reduced initial manganese retention of approximately 40% of the administered oral dose. Human infants also have higher retention of ingested manganese during the early neonatal period.[111,112] Manganese retention in formula-fed term infants was approximately 20%.[112] The developing brain takes up a significant proportion of the manganese retained by the body during the early neonatal period. For example, Keen and coworkers report that approximately *87o* of the total oral manganese dose is retained in the brain by rats.[110] These age-dependent changes may reflect reduced control by the gastrointestinal and hepatobiliary systems or the lower body burden of manganese present in neonatal animals.

Respiratory-Tract Absorption

There are a limited number of studies that have evaluated manganese absorption from the lung. No studies were located regarding the absolute amount of manganese that is absorbed by humans or animals after inhalation exposure to manganese dusts. There are a number of factors that influence pulmonary deposition of inhaled manganese particles. Particle size heavily influences the site at which manganese particles are deposited within the respiratory tract. Lung deposition is favored following the inhalation of particles less than 0.1 μm in size as well as those ranging in size from 0.8 to 3 μm. The soluble fraction of manganese particles deposited in the lower airways may be absorbed from the lung, while insoluble components may be cleared or translocated to other sites within the respiratory system. Particle translocation may lead to pulmonary toxicity at those other sites. The rate at which manganese is absorbed from the lung may influence brain and other organ manganese delivery. In a study by Rhoads and Sanders,[113] for instance, a number of metal compounds that were more rapidly absorbed from the lungs were retained for longer time periods at other sites in the body where they could potentially exert their effects. Larger manganese particles that are deposited in the upper airways may be transported by mucociliary transport to the throat where they are subsequently swallowed and a fraction of this ingested manganese can be absorbed. Manganese particles that deposit in the nose can also be absorbed or cleared. One site where nasal absorption occurs is at the olfactory epithelium. Inhaled manganese absorbed by the rat olfactory epithelium can undergo transport along the olfactory nerve to the olfactory bulb.[114-116] Cellular mechanisms involved with manganese absorption within the respiratory tract are unknown.

Absorption of manganese deposited in the lung is influenced by particle solubility. Roels et al.[117] measured blood manganese levels in 3-month-old rats following intratracheal administration of a relatively soluble ($MnC1_2$) or insoluble (MnO_2) form of manganese (administered at 1.22 mg Mn/kg). Peak blood levels occurred following MnC12 instillation within 30 min, whereas a similar dose of MnO_2 did not reach peak blood manganese levels until 7 days following instillation, suggesting that the insoluble form is less bioavailable. Manganese levels in blood following $MnC1_2$ and MnO_2 dosing reached a maximal level of 7050 ng/dl at 30 min and 760 ng/d1 168 h following administration, respectively. Roels et al.[117] also showed that more soluble forms of manganese (e.g., $MnC1_2$ vs. MnO_2) given to 3-month-old rats via intratracheal instillation are more readily delivered to the brain. Dorman and coworkers[118] showed that lung manganese concentrations were higher in rats exposed to a soluble form of manganese (e.g., $MnSO_4$) when compared with levels seen following inhalation of considerably less soluble manganese particles like the phosphate or tetroxide forms. This finding is consistent with enhanced pulmonary absorption of manganese from the more soluble $MnSO_4$ particle. Moreover, animals exposed to high levels (3 mg Mn/m^3) of $MnSO_4$ have significantly higher striatal, testes, and liver manganese concentrations when compared with levels achieved following exposure to the insoluble tetroxide (Mn_30_4) form. These studies confirmed that brain uptake of inhaled manganese is similarly influenced by particle solubility.

Dermal Absorption

No studies were located regarding absorption in humans or animals after dermal exposure to manganese. It is generally assumed that uptake of manganese across the intact skin is very limited.[6]

MANGANESE DISTRIBUTION (EXCLUDING BRAIN)

Manganese Transport Proteins in Blood

No unique mammalian transporters are known for manganese. The affinity of divalent manganese toward endogenous ligands is relatively low. It does not avidly complex with sulfhydryl (—SH) groups or amines, and it shows little variation in its stability constants for endogenous complexing ligands such as glycine, cysteine, riboflavin, and guanosine. Within the plasma, approximately 80% of manganese is bound to globulin and albumin, and a small fraction of trivalent manganese is bound to transferrin.[90,91,119] At normal plasma iron concentrations (0.9-2.8 μg/ml), iron-binding capacity (2.5- 4 μg/ml), and transferrin concentration (3 mg/ml, with 2 metal-ion-binding sites per molecule [M_r 77000], of which only 30% are occupied by trivalent iron), transferrin has 50 μmol/L of unoccupied trivalent manganese binding sites, and has, there-fore, been implicated as a potential transporter for manganese across the blood-brain barrier and other membranes (discussed later).[120]

Subcellular Localization of Manganese

At physiological levels, manganese specifically concentrates in mitochondria,[121-123] where it most likely complexes with ATP. With exposure to excess manganese, Liccione and Maines[124] demonstrated a two-fold greater increase in manganese in the mitochondria! fraction of the stritum than that of the whole brain. Brenneman and coworkers[49] did not find selective uptake of manganese in the mitochondrial fraction of the striatum or cerebellum from neonatal rats that were given high doses of manganese (0, 25, or 50 mg $MnC1_2$/kg/day) from birth throughout early adulthood (postnatal day [PND] 1 to 49). In the Brenneman study, selective uptake was defined as an increase in the percentage of total regional manganese in the mitochondrial fraction with exposure to excess manganese. Brenneman et al.[49] concluded that if this percentage is not altered with increased tissue levels of manganese, then the normal distribution of manganese is being maintained, and selective uptake has not occurred. It appears more accurate, in light of the findings re-ported by Brenneman et al.[49] to state that chronic manganese administration increases mitochondrial manganese concentrations but does not markedly change the relative proportion of cellular manganese found in the mitochondrial fraction.[49] Lai et al. reached a similar conclusion for a wide range of brain subcellular fractions.[125]

Systemic Manganese Distribution

Manganese is found in all mammalian tissues with concentrations ranging from 0.3 to 2.9 μg manganese/g wet tissue weight and there is little variation among species with regard to tissue manganese concentrations (Table 2).[41,53] Tissues rich in mitochondria and pigments (e.g., retina, dark skin) tend to have high manganese concentrations. Bone, liver, pancreas, and kidney typically have higher manganese concentrations than other tissues. The largest tissue store of manganese is in the bone. Tissue manganese concentrations in normal and manganese-exposed individuals are discussed in greater detail later in this review.

Manganese Distribution Across the Placenta

In vitro studies have suggested that the human placenta accumulates manganese and that the bidirectional transfer of manganese across the placenta is low.[126] In vivo rodent studies using oral gestational exposure have further demonstrated that the amount of manganese that crosses the placenta is low.[127] Despite this apparent barrier, fetotoxicity (reduced fetal body weight or structural anomalies) in the absence of maternal toxicity has been reported to occur in mice exposed orally to high levels of manganese.[128-130]

Few studies have examined fetal manganese tissue concentrations in humans. Casey and Robinson (1978)[131] reported that manganese concentrations in a variety of tissues obtained from 40 human fetuses ranged from 0.35 to 9.27 μg Mn/g dry weight. In vivo studies in laboratory animals have shown that ingested manganese can accumulate in the fetal brain following gestational exposure.[50] Placental delivery of manganese also occurs in rats following inhalation exposure with elevated fetal liver (but not brain) manganese concentrations being observed (Dorman, et al., in press *[Neurotoxicology]).*

TABLE 2
Tissue manganese concentration (μg/g of wet tissue) from presumed normal adult subjects (Mean ± SD)

	Tissue manganese concentration (μg/g)		
Tissue	Humans	Rats	Monkeys
Bone	0.14 ± 0.06[b]	0.47 ± 0.24[e]	0.57 ± 0.26[j]
Cerebellum	1.67 ± 0.50[b]		
	0.52 ± 0.42[d]		
Globus pallidus	0.39 ± 0.05[c]	0.55 ± 0.20[i]	1.70 ± 0.27[j]
	1.93 ± 0.89[b]		
Hippocampus	1.06 ± 0.33[b]		
Heart	0.89 ± 0.25[b]		0.35 ± 0.11[k]
	0.54 ± 0.43[d]		
Liver	4.04 ± 1.59[b]	2.72 ± 0.42[e]	1.46 ± 0.77[k]
		0.31 ± 0.07[f]	2.72 ± 0.84[l]
		2.91 ± 0.70[g]	
		2.14 ± 0.40[i]	
Lung	0.48 ± 0.31[d]	0.32 ± 0.09[e]	0.17 ± 0.14[k]
		0.36 ± 0.38[g]	0.27 ± 0.14[l]
		0.18 ± 0.04[i]	
Olfactory bulb	3.36 ± 0.69[a]	1.24 ± 0.63[e]	
Pancreas	1.0 ± 0.77[d]	1.4 ± 0.2[h]	1.41 ± 0.15[k]
Putamen	0.43 ± 0.11[c]	0.44 ± 0.08[i]	1.83 ± 0.20[j]
	2.08 ± 0.72[b]		0.47 ± 0.09[k]
Kidney	4.98 ± 1.67[b] (renal cortex)	0.19 ± 0.06[f]	0.92 ± 0.11[k]
	3.32 ± 1.41[b] (renal medulla)	0.92 ± 0.38[g]	1.03 ± 0.24[l]
		0.93 ± 0.12[l]	
Skeletal muscle	0.31 ± 0.16[b]		0.19 ± 0.07[k]
	0.31 ± 0.26[d]		
Spleen	0.39 ± 0.59[d]	0.31 ± 0.44[g]	0.27 ± 0.12[j]
			0.24 ± 0.12[l]
Striatum		0.46 ± 0.03[e]	
Substantia nigra	1.06 ± 0.68[b]		
Testes		0.40 ± 0.09[e]	
		0.19 ± 0.09[f]	
		0.25 ± 0.11[i]	

[a] Bonilla et al. (1982).[223]
[b] Bush et al. (1995).[112]
[c] Tracqui et al. (1995).[222]
[d] Yukawa et al. (1980).[221]
[e] Dorman et al., 2001 (10-ppm diet).[93]
[f] Rehnberg et al., 1980 (6-month).[41]
[g] Ulrich et al., 1979.[232]
[h] Kodama et al., 1991.[201]
[i] St-Pierre et al., 2001.[281]
[j] Bird et al., 1984.[235]
[k] Coulston and Griffin, 1977.[236]
[l] Ulrich et al., 1979.[232]

The mechanism involved in manganese distribution across the placenta is unknown; however, both transferrin and DMT-1 may be involved. Data in support of this hypothesis include the findings that transferrin and DMT-1 are present in the placenta and that placental transferrin and DMT-1 are upregulated in maternal iron deficiency, thus minimizing the severity of fetal anemia.[132] Considering the potential shared function of DMT-1 and transferrin in transport of iron and manganese, maternal iron deficiency may lead to enhanced placental delivery of manganese to the fetus. DMT-1 is found in the developing brain, and DMT-1 mRNA has been shown by in situ hybridization to localize to the rat striatum, cortex, hippocampus, and cerebellum at an early gestational age.[133]

MANGANESE DISTRIBUTION TO THE CNS

Regional Delivery of Manganese Within the Brain

The use of MRI techniques has revealed that manganese-poisoned humans and macaque monkeys develop elevated striatum, globus pallidus, and substantia nigra brain manganese concentrations.[5,46,47,134] In contrast, experimental studies have failed to clearly establish that the rodent striatum and globus pallidus preferentially accumulate manganese following high-concentration manganese exposure (see Brenneman et al. for review[49]).

Mechanisms Involved With the Delivery of Manganese Across the Blood—Brain Barrier

A limited number of studies have addressed the transport kinetics of manganese from the blood into the CNS. Collectively, these studies suggest that manganese enters the brain from either across the cerebral capillaries and/or the cerebrospinal fluid (CSF; via choroid plexus transport), or via the olfactory nerve. Each of these transport scenarios is discussed in detail later. At normal plasma concentrations, transport across the capillary endothelium predominates, whereas at high plasma concentrations, transport across the choroid plexus appears more prevalent.[135,136]

Studies using bolus intravenous manganese injections have demonstrated efficient transport of divalent managanese across the blood—brain barrier. These studies showed that manganese transport into the brain was transferrin independent, affected by plasma protein binding, and most likely reflects the transport of manganese as the free ion.[135-139] Collectively these studies suggest that manganese transport across the blood—brain barrier is facilitated by either an active or a passive mechanism governed by a saturable process.[135,139] Since the increase in brain concentrations via blood following a bolus injection is different from that noted via the redistribution from the liver,[138] caution must be exercised in extrapolating from these studies to the contribution of the saturable transport of divalent manganese in physiological conditions. To date, studies on the mechanisms of manganese transport across the blood—brain barrier have focused on transferrin- and DMT-1 mediated transport, but the possibility of transport modulation by other transporters cannot be excluded.

A number of studies substantiate the role of transferrin in manganese uptake across the blood—brain barrier. In vitro, endocytosis of a manganese—transferrin complex in cultured neuroblastoma cells (SHSY5Y) has been noted.[140] A time-dependent increase in ^{54}Mn uptake in the rat brain has also been described,[141] likely reflecting capillary endothelial transport of manganese in the trivalent (Mn^{3+}) oxidation state. However, the internalization of manganese—transferrin complexes into brain capillary endothelium has yet to be demonstrated.

The hypotransferrinemic (hpx/hpx) mouse in which a genetic defect results in <1% of normal plasma transferrin concentration has provided a unique opportunity to examine the role of transferrin in manganese and iron transport into the CNS. The hpx/hpx mouse is the result of a spontaneous mutation, and it has an mRNA splicing defect resulting in virtually no synthesis of transferrin protein.[142] No difference was found in total brain uptake of iron or manganese upon intraperitoneal or intra-venous injections of $^{59}FeC1_3$ or $^{54}MnC1_2$, respectively, between +/+ (controls) and hpx/hpx mice.[143,144] Striking differences in regional distribution were noticed for ^{59}Fe, but only subtle differences in the distribution of brain ^{54}Mn,[145] consistent with the transferrin receptor system being the predominant mechanism for iron delivery to the brain. Slightly lower levels of ^{54}Mn in cerebral cortex and corpus collosum were observed in the hpx/hpx mice relative to +/+ controls. Although transferrin-receptor-mediated transport appears less important for delivery of manganese to the brain in hpx/hpx mice,[146] it must be considered that transferrin-independent mechanisms (such as DMT-1 discussed later) might be unmasked by the genetic defect, thus compensating for hypotransferrinemia. Consistent with this possibility are studies by the same authors[144] in which transferrin was required for normal distribution of ^{59}Fe and ^{54}Mn in brains of "normal" mice.

Transferrin receptors have been identified at the surface of the cerebral capillaries.[147] Manganese may be transported from high-density transferrin receptor expressing regions[148] by means of axonal transport. Indeed, the nucleus accumbens and the caudate-putamen, two brain nuclei that are abundantly rich in transferrin receptors, provide efferent fibers to areas that are rich in manganese, such as ventral-pallidum, globus pallidus, and substantia nigra. Manganese can also be transported along nerve fibers (by means of axonal transport) in the olfactory pathway, optic nerve, and brain parenchyma.[114,149-153]

There is growing evidence that DMT-1 is involved in brain manganese delivery.[154] In the microcytic anemia (mk) mouse and the phenotypically similar Belgrade (b) rat,[155-157] orthologous mutations (glycine 185 to arginine) in the DMT- I gene result in significantly reduced dietary iron absorption. The role of the defective DMT- I allele in the transport of manganese across the blood-brain barrier has been recently evaluated in homozygous Belgrade (b/b) rats that exhibit hypochromic anemia and heterozygous (-Fib) Belgrade rats.[158]

Plasma clearance and up-take by the CNS after intravenous injection of radioactive ^{54}Mn bound to transferrin or mixed with serum have demonstrated that plasma clearance of manganese-transferrin was much slower than manganese-serum, but both were faster than the clearance of iron-transferrin. Uptake of ^{54}Mn, as well as ^{59}Fe by the brain was less in b/b than +/b rats, suggesting that the defective DMT- I allele affects the distribution of both metals, and that manganese and iron might share DMT- 1 transporters in the blood-brain barrier.[158]

While the majority of studies suggest that manganese and iron compete for the same carrier, it should be also pointed out that manganese and iron transport from the plasma to the brain has been postulated to be synergistic rather than competitive in nature, and that excessive intake of iron plus manganese may accentuate the risk of tissue accumulation of the second meta1.[159] These studies suggest that manganese dosimetry is complex and that multiple pathways are involved in delivery of manganese to the brain.

Manganese Delivery Across the Choroid Plexus

The choroid plexus is potentially an important site for brain delivery of manganese. This structure is where injected ^{54}Mn first appears in the rodent brain.[145,153] The regulation of substrate entry into the brain via choroid plexus synthesis of cerebrospinal fluid (CSF) is different from that at the blood-brain barrier. Capillaries in the choroid plexus are fenestrated, and substances must first be taken up by choroid plexus epithelium. This epithelium then secretes CSF. Eventual neuronal uptake of substances from CSF must proceed via the ependymal cells. It is thought that tanycytes of the ependyma might play an important role in delivery of non-transferrin bound iron to the hypothalamus.[160] London et al.[161] reported a rapid localization of manganese in the choroid plexus observed on MRI; similarly, radiotracer studies of manganese injected into the intracerebro-ventricular space revealed that radiolabeled manganese was located in the choroid plexus within 1 h and was located in the rat dentate gyrus and CA3 of the hippocampus 3 days postdosillg.[149] Murphy et al.[135] measured the kinetics of manganese transport in the brains of adult male rats following intravenous infusion with $^{54}MnCl_2$. This experiment revealed that transport of Mn^{2+} into the choroid plexus was dependent upon a saturable mechanism. At all plasma manganese concentrations tested (from 0.8 to 78 nmol/ml), the transfer coefficient for manganese uptake into the choroid plexus was significantly higher than in any other area of the CNS. For example, at 0.08 nmol/ml, the transfer coefficients for the CSF and the choroid plexus were $16.2 \pm 2.43 \cdot 10^{-6}$ ml/sec x g and $23,800 \pm 2910 \cdot 10^{-6}$ ml/sec x g, respectively. Even after correcting for differences in compartment size, influx of manganese into the choroid plexus was an order of magnitude greater than influx into CSF.

Olfactory Nerve Delivery of Manganese

The olfactory system forms a direct interface between the nervous system and the external environment. Certain xenobiotics can bypass the systemic circulation and gain access to the brain and/or CSF directly following nasal administration.163 Three major pathways could facilitate the movement of a xenobiotic from the nose to the brain, including (1) an olfactory nerve pathway, (2) an olfactory epithelial pathway (independent of the olfactory receptor neuron resulting in transport to the brain along the perineuronal space around the olfactory nerve), and (3) a systemic pathway secondary to movement from the nasal epithelium to the blood in the submucosal space.[163]

Recent research results suggest that direct intraaxonal transport of manganese must be considered when evaluating brain delivery of inhaled manganese (reviewed by Tjiilve and Henriksson[164]). Olfactory transport of manganese occurs in the rat, mouse, and freshwater pike following intranasal instillation.[149,164,165] After intranasal instillation, manganese can migrate to most parts of the brain through its ability to jump neuron synapses and travel along secondary and tertiary neurons.[166] Elevated brain concentrations of manganese were detected 12 h following one-sided intranasal instillation of manganese chloride by Gianutsos et al.[149] With multiple injections, these investigators were able to see increased manganese concentrations in the striatum as well, demonstrating that manganese is able to reach the striatum under certain exposure conditions.[149] Dose dependency of the transport of manganese from the olfactory epithelium to the olfactory bulb was further demonstrated following intranasal administration of manganese chloride.[150] This propensity to travel along secondary or tertiary neurons noted with manganese is not observed with cadmium and other metals.[164]

Until recently, little was known regarding the olfactory trans-port of manganese following inhalation exposure. Brenneman and coworkers[114] and Dorman et al.[115] conducted studies in rats using short-term (90-min) inhalation exposure to either a water-soluble ($^{54}MnCl_2$) or insoluble ($^{54}MnHPO_4$) manganese aerosols. These investigators used an animal model in which one nostril was occluded, thus restricting olfactory transport of manganese to the side of the rat brain on the other side to the patent nostril. In these studies, direct delivery along the olfactory route accounted for nearly all the ^{54}Mn found in the olfactory bulb and tract of the rat brain following acute $^{54}MnCl_2$ or $^{54}MnHPO_4$ inhalation.

Similar aerosol concentrations (0.54 vs. 0.39 mg Mn/m^3 for the $^{54}MnCl_2$ and $^{54}MnHPO_4$ exposures, respectively) and particle sizes (2.51 vs. 1.68 μm for the $^{54}MnCl_2$ and $^{54}MnHPO_4$ exposures, respectively) were used in these studies. Manganese clearance from the olfactory epithelia was slower following exposure to manganese phosphate when compared to the more soluble chloride salt.[115] In addition, a smaller fraction of the ^{54}Mn initially deposited on the olfactory epithelium was found in the olfactory bulb one day after the end of the $^{54}MnHPO_4$ exposure. The finding of decreased olfactory epithelial clearance and reduced olfactory bulb delivery following manganese phosphate inhalation is consistent with the results of longer term (2-week) inhalation experiments with soluble and insoluble manganese salts.[115] Dorman and coworkers[115] showed that inhalation exposure to soluble forms of manganese (e.g., manganese sulfate) resulted in higher olfactory bulb manganese concentrations than those achieved following exposure to a relatively insoluble form of manganese (e.g., manganese phosphate or tetroxide). Thus, the olfactory route may be a significant pathway by which manganese gains access to certain regions within the rat brain.

Fechter et al.[116] examined whether particle size could influence brain delivery of inhaled manganese. These investigators exposed juvenile Long-Evans rats to an insoluble form of manganese (MnO_2). Inhalation exposures were conducted for 6 h/day, 5 days/week, for 3 weeks. Fechter et al.[116] exposed animals to either air or MnO_2, at 3 mg Mn/m^3. Two different-sized manganese particles were used (mass median aerodynamic diameter [MMAD] of 1.3 and 18 μm) in this experiment as well. End-of-exposure olfactory bulb manganese concentrations were approximately 1.5, 2.6, and 1.6 μg/g for rats exposed to either air, fine particles (1.3 μm), and coarse particles (18 μm), respectively. Although olfactory-bulb concentrations were elevated in rats exposed to the 1.3-μm particles, this increase was not statistically significant. Results of Fechter et al.[116] suggest that particle size may influence delivery of manganese to the rat olfactory bulb because manganese delivery appeared to be smaller for the large particles (18 μm). This study must be interpreted with caution; however, since Fechter et ai.[116] also showed that lung manganese concentrations were approximately 2.5 and 1.6 μg Mn/g for rats exposed to the 1.3- and 18-μm particles, respectively. Although marginally inhalable, an 18-μm particle would not be expected to be deposited beyond the nasopharynx in the rat.[168,169] The lung manganese concentrations reported in this study following exposure to the 18-μm particle are therefore inconsistent with the particle size distribution presented by these investigators.

The toxicological significance of olfactory transport of manganese remains controversial. Although olfactory transport rapidly delivers manganese to brain structures in the olfactory pathway, it is relatively slow (or incapable) at delivering manganese to the striatum and other more distant brain structures.[114,115,166] Henriksson and Tjalve[167] found that intranasal instillation of manganese results in alterations in olfactory bulb expression of glial fibrillary acidic protein and S- 100b in rats. These proteins are known markers of damage to astrocytes, an important support cell found within the CNS. Manganese exposure by humans has been associated with olfactory deficits (manganese-enhanced olfactory sensitivity); whether these alterations are linked to direct olfactory uptake remains unknown.[7,170]

The relevance of nasal uptake studies conducted in rats to human manganese inhalation exposure is unknown. Significant interspecies differences in nasal and brain anatomy and physiology exist.[171] In the rat, the olfactory bulb accounts for a relatively large portion of the CNS, and the nasal olfactory mucosa covers approximately 50% of the total nasal epithelium. These structures are proportionately smaller in primates; for example, the olfactory mucosa covers approximately 5% of the total nasal epithelium in humans. These anatomical

differences argue that this route of brain delivery may be less important in humans as compared to the rat. In addition, total airflow to the olfactory mucosa is slightly lower in humans than in rats.[172,173] These differences may predispose the rat, more so than humans, to olfactory deposition and potential olfactory transport of manganese; however, direct experimental evidence in support of this conclusion is lacking. Furthermore, the experimental animal inhalation studies that have been conducted to date are short-term, relative to the chronic exposures of occupational and nonoccupational populations.

Factors That Influence Brain Manganese Delivery

Consistent with the hypothesis of shared transporters for manganese and iron are observations that plasma iron over-load significantly decreases brain manganese levels.[120,174,175] Conversely, brain manganese levels are elevated with decreased plasma iron concentrations.[144,176-178]

A recent study performed by the Aschner laboratory[176] examined the effects of both marginal iron deficiency (ID) (in the absence of anemia) and manganese fortification (CNMn+ diet) on manganese accumulation in the rat brain. Hemoglobin and hematocrit levels (141 g/L and 40To, respectively) revealed that the iron deficient rats were not anemic; however, plasma transferrin concentrations were indicative of iron deficiency (i.e., significantly higher than controls). A significant increase in manganese concentrations across brain regions in iron-deficient rats consuming high manganese diet (100 ppm; IDMn +) was noted. Highly fortified manganese diet (100 ppm) in iron-replete rats, and "normal" manganese diet (10 ppm) in iron-deficient animals did not lead to changes in brain manganese levels com-pared with controls. These studies are consistent with increased brain manganese accumulation (in rodent experimental models) in conditions of iron deficiency.

The route of exposure can influence the delivery of manganese to the CNS.[12,117] Roels et al.[117] investigated brain manganese concentrations in rats following exposure to either a soluble ($MnCl_2$) or insoluble (MnO_2) form. These chemicals were administered via intratracheal injection (as a surrogate for in-halation) or by gavage (i.e., oral administration). This experiment was designed in order to achieve similar blood manganese concentrations and was thus designed to account for low oral absorption of manganese versus the higher rate of absorption from the lung. When administered intratracheally once a week for 4 weeks, 1.22 mg manganese/kg as $MnCl_2$ resulted in a 68% steady-state increase in blood manganese concentration after the dosing period. This dose also resulted in significantly increased concentrations of manganese in the rat striatum (205% increase) and cortex (48% increase) when compared to control rats. Ad-ministration of manganese as $MnCl_2$ by gavage (administration of 24.3 mg manganese/kg as $MnCl_2$ once weekly for 4 weeks) caused roughly the same amount of increased manganese in the blood (68% increase vs. controls) as intratracheal administration of manganese in the same form, but it did not cause as significant an increase of manganese in the cortex (22% increase vs. controls).[117] Striatum manganese concentrations were unaffected following gavage administration of manganese. Thus, pulmonary delivery of manganese appears to be more efficent than ingestion in increasing manganese concentration in the brain.

Pharmacokinetic factors that may contribute to the increased efficiency in brain manganese delivery observed after inhalation exposure include increased manganese absorption from the respiratory tract, slower blood clearance of absorbed manganese, and direct olfactory transport of manganese to the brain.[12]

MANGANESE TRANSFORMATION

Manganese does not undergo metabolism; as its elemental form, it is absorbed and excreted unchanged. However, manganese can exist in a number of oxidation states, and it can undergo changes in its valence states within the body. Measurements of unpaired electrons in Mn^{2+}, Mn^{3+}, and Mn^{4+} complexes found in tissues and fluids using electron spin resonance (ESR) has been used to evaluate valence forms of manganese in biological media. When animals were injected with divalent manganese (as $MnCl_2$), levels of manganese increased in bile and tissues, but only a small portion of this was in a form that gave an ESR signal.[179-180] This finding suggests that Mn^{2+} is converted to another oxidation state (probably Mn^{3+}), but it is also possible that formation of complexes between Mn^{2+} and biological molecules (bile salts, proteins, nucleotides, etc.) results in loss of the ESR signal without formal oxidation of the manganese ion. The form of manganese in most enzymes is Mn3+,

while most manganese taken into the body exists as either the Mn^{2+} or Mn^{4+} forms. Mn^{2+} is the predominant form in biological systems.

Gibbons et al.[181] reported that human ceruloplasmin led to the oxidation of Mn^{2+} to Mn^{3+} in vitro. This pathway has been considered a possible mechanims for manganese oxidation in blood. Gibbons et al.[181] also reported that manganese oxidation led to a shift in the in vitro binding of manganese from α2- macroglobulin to transferrin. The valence state of manganese may also influence its disposition in the body. Komura and Sakamoto[182] showed that distribution of an insoluble tetravalent (e.g., MnO_2) and soluble divalent ($MnCl_2$) forms of manganese to the cerebral cortex differed following manganese ingestion. They found that less soluble forms such as MnO_2 were taken up to a significantly greater degree in cerbral cortex than the more soluble $MnCl_2$ form. However, these investigators showed that the corpus striatal binding characteristics of the +4 valence state of manganese in MnO_2 were not substantially different from those of the divalent forms in $MnCl_2$. Roels et al.[117] also found differences in manganese concentrations in blood and brain regions depending on the initial oxidation state of the metal. Manganese metabolism or initial valence states may be an important determinant of manganese retention in the body.[6]

The valence of manganese and hence its metabolism may also influence the toxicity of manganese. In vitro Mn^{3+} appears to be more cytotoxic than Mn^{2+} species, possibly due to higher oxidative reactivity and a closer radius resemblance to iron.[183] The enhanced ability of trivalent manganese to induce oxidative stress has been confirmed in rats given either manganese chloride (Mn^{2+}) or manganese acetate (Mn^{3+}).[184] Oxidation of important cellular components by trivalent manganese (Mn^{3+}) might represent the primary means by which manganese mediates cellular damage. However, it is currently unknown how much intracellular (and intramitochondrial) manganese is in the divalent versus trivalent forms.

MANGANESE EXCRETION

Estimation of Manganese Excretion Rates

Administration of a ^{54}Mn tracer can be used to determine whole-body elimination of manganese and indirectly assess liver manganese metabolism. In animals and humans, the whole-body elimination of a single tracer dose of ^{54}Mn is biphasic,[92,95,97] and the rates of elimination can be altered by dietary and inhaled manganese intake rates.[93] The rapid-phase elimination rate constant and the proportion of the tracer dose eliminated in the rapid phase increases with increasing rates of manganese ingestion.[92,185] Dose-dependent elimination of a trace dose of ^{54}Mn has been observed in manganese-exposed miners when compared to occupationally unexposed humans.[186]

Biliary Excretion

Biliary excretion is the main pathway by which manganese reaches the intestines where most of the element is ultimately excreted in the feces.[86,96] Regardless of manganese intake, adult humans generally maintain stable tissue levels of manganese. The basis for this homeostatic mechanism is the regulation of both absorption and excretion rates for manganese.[6] Absorbed manganese is removed from the blood by the liver and is excreted into the intestine via the bile. Some of the manganese in the intestine is reabsorbed through enterohepatic circulation.[187] The rate of biliary excretion of manganese is influenced by a number of factors.

Lee and Johnson[188] reported that the rate of ^{54}Mn excretion was significantly accelerated in rats by increased dietary manganese concentrations, while the efficiency of ^{54}Mn absorption was decreased by increasing dietary manganese concentrations. Thus, the fraction of ingested manganese retained by the body is regulated in order to maintain normal tissue manganese concentrations under different dietary conditions.

Dorman et al.[118] and Vitarella et al.[189] also showed that enhanced biliary manganese excretion occurs in rats following repeated manganese inhalation. Dorman et al.[118] exposed male CD rats to either $MnSO_4$ or Mn_3O_4 at 0, 0.03, 0.3, or 3 mg Mn/m^3 for 6 h/day for 7 days/week (14 exposures). End-of-exposure bile manganese concentrations and whole-body ^{54}Mn elimination were then determined. Elevated bile manganese concentrations and increased whole-body ^{54}Mn clearance rates were observed in rats exposed to high

concentrations (3 mg manganese/m3) of either Mn_3O^4 or $MnSO_4$. These results are consistent with those of Cotzias et al.,[186] who reported dose-dependent manganese elimination in miners exposed to manganese dusts.

Biliary excretion is reduced in neonatal animals and, as we discussed earlier, exposure during this period of development may result in increased delivery of manganese to the brain and other tissues. For example, in mice, rats, and kittens, there is an almost complete absence of biliary manganese excretion during the neonatal period.[190] However, data in neonatal rats indicate that manganese retention rates decrease around postnatal day 12 to 16 to approximate rates observed in adult animals. This is indirect evidence that biliary manganese excretion may mature during the end of the neonatal period, though the exact time frame across species is unknown, or that stores become sufficient and manganese elimination in bile is activated.

Liver disease is a risk factor for increased accumulation of manganese in the CNS, in both animal models and hUManS.[145,191,192] Patients with cholestatic liver disease with biliary atresia (associated with diminished biliary excretion of manganese) display hypermanganesemia and T1 -weighted MRI signal hyperintensity in the globus pallidus.[193-195] Analogous MRI findings have been reported in the globus pallidus of cirrhotic patients with subclinical hepatic encephalopathy.[196-198]

Liver disease can also alter manganese excretion rates following inhalation exposure. For example, Salehi et al.[199] determined brain manganese accumulation in a rat model of liver disease (end-to-side portacaval anastomosis) following inhalation exposure to manganese phosphate (3.05 mg Mn/m^3 for 8 h, day, 5 days/week for 4 consecutive weeks). Salehi et al.[199] showed that brain manganese concentrations in the cerebellum, frontal cortex, and globus pallidus were significantly higher in the manganese-exposed group compared to the control group. This study lacked a concurrent manganese-exposed sham surgery control group; thus, this study does not clearly demonstrate whether there is the potential for increased manganese brain accumulation in patients with compromised liver function.

Manganese exposure can also exacerbate liver dysfunction. For example, an intravenous bolus of bilirubin followed by manganese causes cholestasis in rats.[200] Therefore, liver function is an important consideration in the management of individuals with significant manganese exposure.

Pancreatic Excretion

The pancreas accumulates manganese. Studies conducted in rats have shown that pancreatic excretion of manganese accounts for only a small fraction of the absorbed manganese dose.[86] Kodama et al.[201] have shown that manganese distributed to the pancreas is bound to pro-carboxypeptidase B. Manganese can be excreted in pancreatic fluids. Miller et al.[202] showed that concentrations of manganese in bile and pancreatic juices were approximately 0.18 and 0.36 μmol/L, respectively. Ishihara and Matsushiro[203] showed that mean (± SEM) manganese concentrations in pancreatic juices collected from 19 human subjects was 66 ± 13.3 μmol/ml. These investigators estimated that daily manganese excretion via the pancreas was approximately 0.1 to 0.13 μmol Mn/day (assuming a pancreatic juice flow rate of 1.5 to 2 L/day). Ishihara and Matsushiro[203] estimated that manganese elimination in human urine and bile was approximately 0.018 and 0.18 to 0.36 μmol Mn/day, respectively.

Urinary Excretion

As noted earlier, urinary excretion of manganese is generally low. Mean (± SD) urinary manganese concentrations observed in healthy subjects living in northern Italy were 1.02 ± 0.05 pg/L[205] (Table 3). Paschal et al.[206] reported that a large sample (n = 496) of residents of the United States had a mean urine manganese concentration of 0.89 μg/L (25th and 95th percentiles were <0.2 and 3.33 μg Mn/L, respectively) (Table 3). Regression analysis demonstrated a gender-based correlation; however, urine manganese concentration was not correlated with the age of the subject.[206] Greger et al.[207] reported that urinary excretion of manganese by healthy male and female subjects was approximately 0.39 and 0.52 μg Mn/g creatinine/day, respectively.

TABLE 3
Manganese concentration in whole blood, plasma, serum, and cerebrospinal fluid (CSF) of males and females >18 years of age (range 4–14 μg/L in whole blood; 1–30 μg/L in plasma/serum)

Investigator	Country	Analysis method	Study description and number of subjects	Plasma,[a] serum,[b] CSF,[c] or urine[d] manganese (μg Mn/L)
Kristiansen et al. (1997)[282]	Denmark	ETAAS	Men and women aged 40–70 yr (188)	9.08 ± 2.81 (whole blood)
Basun et al. (1991)[283]	Sweden	EDXRF	Elderly M/F normal (25)	5.76 ± 2.94[e]
			DAT (12) aged 75 ± 8 yr	3.79 ± 1.85
Molina et al. (1998)[284]	Spain	AAS	Elderly AD (26) aged 73 ± 8 yr	1.03 ± 0.68[b]
				0.8 ± 0.75[c]
Molina et al. (1998)[284]	Spain	AAS	Normal (28) aged 71 ± 7 yr	1.31 ± 0.63[b]
				1.16 ± 1.7[c]
Jimenez-Jimenez et al. (1998)[285]	Spain	AAS	Elderly PD (37) aged 66 ± 9 yr	0.93 ± 0.9[b]
				1.2 ± 0.98[c]
			Elderly normal (37) aged 62 ± 2 yr	1.22 ± 0.59[b]
				0.88 ± 0.8[c]
Takagi et al. (2002)[77a]	Japan	GFAAS	Adults receiving HPN (12)	27.5 ± 13.8[a,f]
				41.25 ± 22** (whole blood)
			Control (no known disease) (25 M, 21 F)	30.25 ± 11[a,f]
				13.75 ± 5.5** (whole blood)
Rükgauer et al. (1997)[242]	Germany	ETAAS	Adults 22–75 yr (68)	0.79 ± 0.3[a,e]
Diaz et al. (2001)[244]	Spain	GFAAS	Subjects 6–75 yr (368)	1.06 ± 0.62
Baldwin et al. (1999)[286]	Canada	GFAAS	Men (141)	7.0 (whole blood)
			Women (156)	7.9 (whole blood)
Paschal et al. (1998)[206]	United States	ICP-MS	Adults (496)	0.89[d]
Minoia et al. (1990)[205]	Italy	AAS	Adults (1120)	1.02[d]

Note. Data are mean ± SD. GFAAS, graphite furnace atomic absorption spectrophotometry; ETAAS, electrothermal atomic absorption spectrophotometry; AAS, flame atomic absorption spectrophotometry; EDXRF, energy-dispersive x-ray fluorescence; DAT, dementia of Alzheimer's type; AD, Alzheimer's disease; PD, Parkinson's disease; HPN, home parenteral nutrition; CSF, cerebrospinal fluid.

[a,b,c,d] In the last column, these letters refer to the corresponding fluid in the column heading.

[e] Converted from nmol/L.

[f] Converted from μmol/L.

[g] It is unclear why the values seen in this study are substantially different from those reported in the others. The potential for erroneous analysis should be considered.

There is conflicting literature as to whether urinary manganese responds to increased dietary or inhalation exposure to manganese. Greger et al.[207] and Davis and Greger[208] failed to demonstrate a correlation between urinary manganese concentration and the dietary intake of manganese in adult male and female volunteers. Urinary excretion of manganese following inhalation exposure is also a poor biomarker of exposure.[12] For example, Vitarella and coworkers [189] exposed rats by inhalation for 6 h/day for 2 weeks (10 or 14 exposures) to manganese phosphate at 0, 0.03, 0.3, or 3 mg Mn/m^3. Immediately after the end of the inhalation exposure, rats were given an intravenous tracer of ^{54}Mn, and 24-h fecal and urinary manganese excretion was determined during a 16-week postexposure interval. In this study, less than 1% of the administered ^{54}Mn tracer could be detected in the urine, and urinary manganese excretion was not influenced by inhalation exposure. It has been reported that chronically exposed male workers have urine manganese levels that were significantly higher than unexposed persons. For example, male foundry workers had a mean manganese level of 5.7 μg/L, compared to 0.7 μg/L in unexposed controls.[209] Other studies have reported significantly increased levels of urinary manganese in men occupationally exposed to airborne manganese dusts and fumes.[193-195] For example, Lucchini et al.[29] noted a correlation between manganese urine concentration and cumulative manganese inhalation exposure index for workers tested while currently exposed to manganese, but this association was no longer

present when urine samples were taken > 13 days after cessation of exposure. Mergler et al.,[7] however, did not report a significant difference in urinary manganese levels between the exposed and control groups in their occupational study.

Urinary manganese concentrations may be sensitive to manganese depletion. For example, Friedman et al.[16] showed that a patient maintained on a low-manganese diet (providing 0.11 mg Mn/day) had markedly reduced urinary excretion of manganese following 35 days of dietary manipulation (e.g., decreased from 8.6 μg Mn/day to 0.4 μg Mn/day during this time interval).

Excretion Into Milk

Manganese is also excreted in milk (see Table 1). Manganese concentrations found in human and cow's milk can vary dramatically with published ranges of 3-120 and 30-50 μg/L, respectively.[53,55-57] Manganese levels found in rat milk are considerably higher, and they vary during lactation, with the highest concentrations (~330 μg/ml) found in milk produced during the immediate postnatal period.[210] Manganese content in human milk also varies with lactation.[56-58] For example, Stastny and coworkers[56] reported that mean (±SD) human milk manganese concentrations in the fourth week of lactation were 6.6 ± 4.7 μg/L and these levels were significantly higher than those collected during the 12th week of lactation (3.5 ± 1.4 μg/L) (Table 1).

Elimination in Hair

Hair can also accumulate high amounts of manganese, and it has been suggested that hair manganese concentrations may reflect manganese status.[211] Bush et al.[212] reported hair manganese concentrations in 30 human subjects at 1.44 ± 1.34 μg/g wet tissue. Woolf et al.[33] have also reported increased hair levels of manganese in a child with chronic manganese exposure from drinking water. The hair manganese level in his asymptomatic brother was elevated, while blood levels were within the normal range. These studies have suggested that hair manganese could serve as a useful biomarker of exposure. Some studies have found a correlation between exposure level and manganese concentration in hair.[211] Several studies have also found higher manganese levels in the hair of learning disabled children than in non-disabled children.[211,213] Increased manganese levels were also found[214] in the hair of exposed workers (3.2 times higher than matched controls). Other studies, however, have found no correlation between individual hair levels and the severity of neurological effects in manganese-exposed persons.[215] However, interpretation of increased manganese content in hair must be viewed with caution since manganese may be more readily found in darker colored hair[216-217] and dye, bleaching, or other topical treatments may contaminate hair. The suitability of manganese analysis in hair for biomonitoring purposes is subject to great background variation and inherent analytical problems.[218]

Clearance of Manganese From the CNS

Manganese that is delivered to the brain is eliminated over time. Takeda and coworkers[219] reported a biological half-life of manganese in adult rat brain to be on the order of 51 to 74 days. A very similar elimination half-time of 53 days has been reported in a macaque monkey given manganese chloride via an implanted subcutaneous osmotic minipump.[220] Newland et al.[220] also exposed two cynomolgus monkeys to a tracer dose (0.01 to 0.02 μg Mn) of $^{54}MnCl_2$ given as an aerosol via an endotracheal tube and then evaluated head and chest ^{54}Mn levels by gamma spectrometry. They observed that clearance of ^{54}Mn from the head was slow with an estimated half-life of elimination greater than 220 days. This estimate is likely to be confounded by the relatively slow elimination of ^{54}Mn from the skull and may not reflect brain elimination rates for this metal. Cotzias and coworkers[186] reported brain elimination half-time of 53 days in human beings given intravenous ^{54}Mn tracer doses. Dorman et al.[93] reported that neonatal rats given high oral doses of manganese throughout lactation (from PND 1 through PND 21) had control brain manganese concentrations when assessed at study termination (PND 73). This observation suggests an apparent half-life of elimination of manganese from the young adult rat brain to be on the order of 50 days or less.

TISSUE MANGANESE CONCENTRATIONS

Numerous studies are available that describe manganese concentrations in normal and manganese-exposed laboratory animals and humans (tissues and biological media) (Tables 1-6). Indeed, the biomarker most used for manganese exposure in animal studies is tissue manganese concentrations.[13]

Brain and Tissue Manganese Concentrations in Presumed Normal Humans and in Humans Following Manganese Exposure

Tissue manganese concentrations are occasionally evaluated in human postmortem samples. Bush et al.,[212] Yukawa et al.,[221] and others have reported manganese concentrations in a variety of tissues (Table 2). Tracqui et al.[222] examined brain manganese concentrations in three control subjects, and they reported that globus pallidus, putamen, fifth gyrus temporalis, and thalamus manganese concentrations were 0.39 ± 0.05, 0.43 ± 0.11, 0.27 ± 0.05, and 0.33 ± 0.07 (n = 2) μg/g, respectively. These investigators also reported that a 63- year-old woman that was maintained on total parenteral nutrition for 19 months had higher globus pallidus, putamen, and fifth gyrus temporalis manganese concentrations (0.95, 1.00, and 0.44 μg/g, respectively) than in the presumed normal subjects.

Bonilla and coworkers[223] determined manganese concentrations in multiple brain regions from eight presumed normal people who did not have increased manganese exposure, and showed that the highest concentrations were found in the pineal gland and olfactory bulb. Concentrations found in the olfactory bulb (3.36 ± 0.69 μg/g) were approximately two- to three-fold higher than those found in the caudate nucleus, putamen, and globus pallidus.

Bush et al.[212] reported brain manganese concentrations in five human subjects in the cerebellum, hippocampus, substantia nigra, globus pallidus, and putamen were 1.67 ± 0.50, 1.06 ± 0.68, 1.06 ± 0.33, 1.93 ± 0.89, and 2.08 ± 0.72 μg/g, respectively. Yukawa et al.[221] reported that manganese concentrations in the cerebellum were 0.52 ± 0.42 μg/g (*n* = 12). Cerebral and cerebellar manganese concentrations are also available from Japanese accident victims, and at the time of autopsy they corresponded to 0.25 ± 0.098 and 0.36 ± 0.11 μg Mn/g wet tissue.[224] However, these values are likely lower than those in the striatum and globus pallidus, both of which are known to accumulate more manganese than cerebellum.[225]

A number of studies have examined the relationship between occupational exposure to manganese and brain manganese concentrations (as assessed by MRI imaging). A recent cross-sectional study[226] recently assessed healthy workers (48 male and 27 female) at a dry-cell battery factory. Internal exposure was quantified by the analysis of manganese in the blood. Chronic exposure was defined as a cumulative index (CBI), including duration of exposure, individual workplace factors, and previously measured concentrations of MnO_2 in dust samples.[226] Clinical examinations failed to detect parkinsonisn-like symptoms in any of the tested workers. The mean manganese concentration in blood was 12 μg/L (range 3.9-23.3 μg/L) (Table 4). MRI revealed a significant positive correlation between manganese levels in blood and manganese concentrations in the brain, but no brain atrophy could be detected. An earlier study[227] also reported an increase in signal intensities on the T1-weighted images in asymptomatic manganese-exposed workers compared with manganese nonexposed manual workers and nonexposed clerical workers in the same factories. Both studies were not designed to determine whether progression of manganism or parkinsonism-like syndromes from manganese exposure occurs, and currently this issue remains unresolved.

Additional case-report studies establish that exposure to manganese in welders leads to brain MRI bilateral hyperintensity on T1-weighted images in the globus pallidus and other brain regions.[228,229] A recent study[230] also suggests that a parkinsonism-like syndrome in welders is distinguished clinically only by age at onset, suggesting that potential exposure to manganese fumes may be a risk factor for Parkinson's disease. However, this study did not assess exposure to manganese and a genetic contribution to susceptibility in these exposed individuals cannot be excluded. A population-based case-control study to assess occupational exposures to metals as risk factors for Parkinson's disease suggests that more than 20 years of exposure to manganese is a risk factor for Parkinson's disease.[231]

TABLE 4
Manganese concentration in whole blood, plasma, urine, and hair of exposed workers (range 12–15 μg Mn/L in whole blood)

Investigator	Country	Analysis method	Study description and number of subjects	Whole blood (μg Mn/L)	Urine (μg Mn/g creatinine)	Hair (μg Mn/L)
Dietz et al. (2001)[287]	Germany	AAS	MnO_2-exposed workers (11)	14.8	0.4	5.8
Dietz et al. (2001)[287]	Germany	AAS	MnO_2-unexposed workers (11)	12.2	0.4	5.9
Domingo et al. (2001)[288]	Spain	ICP-MS	Workers in hazardous waste incinerator (28)	15.1 ± 9.0		
Kim et al. (1999)[227]	South Korea	GFAAS	Exposed workers (89)	14.2 ± 5.3		
Kim et al. (1999)[227]	South Korea	GFAAS	Unexposed workers (16)	11.7 ± 3.7		
Roels et al. (1987)[30]	Belgium		Exposed 0.97 mg Mn/m^3	13.6 ± 6.4	4.76	
			Unexposed	5.7 ± 2.7	0.3	
Roels et al. (1992)[31]	Belgium		Exposed 0.179 mg Mn/m^3	8.1	0.84	
			Unexposed	6.8	0.09	
Chia et al. (1993)[26]	Singapore		Exposed 1.59 mg Mn/m^3	25.3	6.1[a]	
			Unexposed	23.3	3.9[a]	
Mergler et al. (1994)[7]	Canada	GFAAS	Exposed 0.032 mg Mn/m^3	11.2	1.07	
			Unexposed	7.2	1.05	
Lucchini et al. (1999)[289]	Italy	AAS	Exposed 0.0967 mg Mn/m^3	9.7	1.81	
			Unexposed	6.0	0.67	
Paschal et al. (1998)[206]	United States	ICP-MS	Adults (496)		0.89	
Alessio et al. (1989)[208]	Italy	AAS	Exposed 0.4–1.1 mg Mn/m^3		5.7[a]	
			Unexposed		0.7[a]	

Note. Data are mean ± SD. GFAAS, graphite furnace atomic absorption spectrophotometry; AAS, flame atomic absorption spectrophotometry; ICP-MS, inductively coupled plasma mass spectrophotometry
[a] μg/L.

Brain and Other Tissue Manganese Concentrations in Presumed Normal Nonhuman Primates and in Nonhuman Primates Following Manganese Exposure

Brain manganese concentrations have also been reported in experimental cases of manganism in nonhuman primates. The most complete information is derived from a study in Indian red-haired monkeys.[185] After 3 months of subcutaneous injections to manganese dioxide (MnO_2) at three dose levels (0, 2.25, 4.5, and 9 g), dose-related increases were noted both in CNS manganese concentrations (20.9, 91.0, 173.7, and 264 μM, in striatum; 35.3, 100.7, 241.7, and 334.4 μM, in globus pallidus, respectively), and manganese-associated clinical signs.

Ulrich and coworkers[232-234] evaluated the toxicity of inhaled manganese oxide (MnO_2) in male and female squirrel monkeys *(Saitniri scuireus)* and Sprague-Dawley rats. Animals were exposed for approximately 21-22 h/day, 7 days/week for 9 months, to either filtered air or atmospheres containing 11.6, 112.5, or 1152 μg manganese/m^3 (MMAD = 0.11 μm, s_g = 3.07). Elevated kidney manganese concentrations were observed in monkeys exposed to either 112.5 or 1152 μg manganese/m^3. Elevated spleen and whole blood manganese concentrations were observed in monkeys exposed to 1152 μg manganese/m^3. Increased lung manganese concentrations were observed in monkeys exposed to either 11.6 or 115 μg manganese/m^3 (an increase was also observed in animals exposed to 1152 μg manganese/m^3; however, this increase was not statistically significant). Brain manganese concentrations were not determined in this study.

Bird and coworkers[235] exposed female rhesus monkeys *(Macaca mullata)* for 6 h/day, 5 days/week for 12 months, to either air or MnO_2 at 30 mg Mn/m^3 (<5 μm particle). Increased putamen and globus pallidus manganese concentrations were observed in manganese-exposed monkeys. Mean (±SD) putamen manganese concentrations observed in the control and manganese-exposed animals were 1.83 ± 0.20 and 3.15 ± 0.13 μg manganese/g wet tissue, respectively. Mean (±SD) globus pallidus manganese concentrations observed in the control and manganese-exposed animals were 1.70 ± 0.27 and 3.04 ± 0.36 μg manganese/ g wet tissue, respectively. Although caudate and substantia nigra manganese concentrations in manganese-exposed monkeys were approximately 134 to 145% of those observed in controls, this increase was not statistically significant

(most likely due to small sample sizes). Bird and coworkers[235] also reported that chronic exposure of monkeys to MnO_2 resulted in a concurrent 34 to 64% decrease in dopamine concentration in the caudate and globus pallidus, respectively.

Coulston and Griffin[236] examined the toxicity of inhaled Mn_3O_4 in rhesus monkeys and rats. Similar to Ulrich et al.,[234] these investigators also generated their manganese aerosol by burning MMT in a natural gas flame. Monkeys were exposed for approximately 23 h/day, 7 days/week for 6 to 15 months, to Mn_3O_4 at 0.1 mg manganese/m^3. An unexposed group of animals was used as controls. One monkey was killed following 6 months of manganese exposure and two animals were evaluated after a 15-month exposure period; these groups were insufficient to allow for statistical evaluations to be performed. Elevated lung, liver, pancreas, kidney, and heart muscle manganese concentrations were observed in the monkeys that were exposed to Mn_3O_4 for one year. Increased pallidum, basal ganglia, cerebellum, and pons manganese concentrations were also observed following the 1-yr inhalation exposure. Mean (± SD) basal ganglia manganese concentrations observed in the control and manganese-exposed animals were 0.50 ± 0.08 and 1.25 ± 0.128 µg manganese/g wet tissue, respectively.

Newland and coworkers[47] exposed a cynomolgus monkey *(Macaca fascicularis)* to a $MnCl_2$ aerosol (20 to 40 mg manganese/m^3 for 2 h/day, 4 days/week) prior to performing MRI evaluations. Brain MRIs were obtained following 3 and 5 months of inhalation exposure. Newland reported that the caudate, putamen, globus pallidus, and pituitary gland selectively took up inhaled manganese.

Brain and Other Tissue Manganese Concentrations in Rodents Following Manganese Exposure

Manganese concentrations in various parts of control rat striatum have been reported to range from 4.4 to 18 µM.[100,117,237] In manganese-exposed rats, the levels of manganese in the striatum increase to 23-70 µM . These levels must be interpreted with caution in light of differential temporal and dosing regimens. In these studies, the manganese was administered in various ways: (I) by adding 0, 1, 10, or 20 mg $MnCl_2$/ml to the drinking water for 120 days of development; [100] (2) by intraperitoneal injection of 1.22 mg manganese (as either MnO_2 or $MnCl_2$)/kg body weight once/week for 4 weeks[117]; (3) or by intrathecal injection of 250 µg $MnCl_2$ into adult rats (250 grams of body weight), and sacrifice 6 h later.[227] As with MRI studies in nonhuman primates,[47] actual manganese levels in the striatum and globus pallidus undoubtedly vary with the time after dosing.

Dorman and colleagues[93,94] examined the impact of dietary or inhalation exposure. In these studies, postnatal day (PND) 10 rats were placed on specially formulated diets that contained 2, 10, or 100 mg manganese/kg diet (ppm). The lowest and highest diets were chosen in order to provide the animals with a marginally deficient or normal level of manganese. The 10-ppm manganese diet used in these studies met current rodent dietary guidelines. After 2 months of dietary manipulation, male rats were exposed by whole-body inhalation for 6 h/day on 14 consecutive days to $MnSO_4$ or Mn_3O_4 at concentrations equivalent to 0, 0.03, or 0.3 mg manganese/m^3. Aerosols with similar particle sizes (MMAD of 1.1 to 1.6 µm) were used in these studies. Rats exposed to 0.092 mg $MnSO_4$/m^3 (0.03 mg manganese/m^3) had elevated lung manganese concentrations when compared to air-exposed male rats. Male rats exposed to 0.92 mg $MnSO_4$/m^3 (0.3 mg manganese/m^3) developed increased striatal, lung, and bile manganese concentrations when compared to air-exposed male rats. Rats given a marginally manganese-deficient diet (2 ppm) had decreased cerebellar manganese concentrations compared with levels observed in rats given the high-normal manganese diet (100 ppm). Male rats given the marginally deficient manganese diet (2 ppm) also developed decreased liver manganese concentrations when compared with animals given the 10 ppm manganese diet. Dorman et al.[93] failed to demonstrate any inhalation-related effects on olfactory bulb, cerebellum, liver, testes, serum, or femur manganese concentrations. Likewise, olfactory bulb, striatum, lung, bile, testes, and serum manganese concentrations were unaffected by dietary treatment despite a 50-fold difference in manganese concentrations used in this study.

Manganese Speciation (Divalent vs. Trivalent Forms)

In nature, manganese can exist in all oxidation states from 2+ to 7+, with divalent manganese being the most relevant to biological conditions. The extent of the neurotoxicity of manganese appears to be determined by its

oxidation state. Manganese is putatively transported into cells in its divalent state and oxidized intracellularly, via reaction with the super-oxide anion to the trivalent state.[238] Manganese in the trivalent state has been linked to cytotoxicity, particularly to dopaminergic cells and other catecholamines in the brain.[238] Furthermore, in the mitochondria, it has been demonstrated that manganese will inhibit complex I thereby leading to altered oxidative phosphorylation.[183,184,238] Trivalent manganese has a stronger affinity for complex I than divalent manganese, but divalent manganese is the most predominant species in vivo. Nevertheless, manganese in any oxidation state will likely spontaneously give rise to some trivalent manganese. HaMai et al.[239] demonstrated that even at trace amounts, trivalent manganese can cause formation of reactive oxygen species. They also showed evidence that divalent manganese fails to induce oxidative effects. While its oxidation state will determine manganese transport and kinetics (e.g., transferrin binds to trivalent manganese exclusively[90,91, 119]), there are no readily available methods for determining the valence of manganese within the body.

BIOMARKERS OF EXPOSURE

Blood Manganese Concentrations

Whole blood, serum, and plasma manganese are the readily available biomarkers of manganese status in humans. An ad-vantage of determining whole-blood manganese concentrations, instead of plasma or serum concentration, is that even slight sample hemolysis can result in artificial increases in serum or plasma manganese concentrations. This effect is due to red blood cell manganese concentrations being 10- to 15-fold higher than that of serum or plasma.[14,240,241] Riikgauer et al.[242] reported that adult humans aged 22 to 75 years had mean (± SD) plasma manganese concentrations of 0.79 ± 0.3 μg manganese/L (14.3 ± 5.7 nmol/L) (Table 3). No correlation was observed between plasma manganese concentration and the age or gender of the adult subject. In general, the available data (Table 3) suggest an apparent age-related decrease in manganese blood concentrations. The implications of this observation for determining age-related levels of manganese, and, by implication, age-related safe exposure levels are unknown.

Children (1 month to 18 years of age) had mean (±SD) serum manganese concentrations of 1.4 ± 0.63 μg manganese/L (25.5 ± 11.4 nmol/L).[242] A negative correlation between serum manganese concentration and the age of the child was observed. For example, mean (±SD) serum manganese concentrations of 2.1 ± 0.81 μg manganese/L and 0.96 ± 0.31 μg manganese/L were observed in infants aged 0 to 6 months *(n* = 13) and children aged 14 to 18 years of age *(n* = 17), respectively (Table 5). Neonates also have blood manganese concentrations that are threefold higher than in adults (Table 5), which may be indicative of increased absorption, reduced excretion, or higher requirement for manganese stores. Whole-blood manganese concentrations from normal children are the highest in neonates, and decrease with age reaching a stable level at 1 year of age (Table 5).[243]

Diaz et al.[244] also reported serum manganese concentrations in a representative sample of citizens living in the Canary Islands *(n* = 368 individuals ranging from 6 to 75 years of age). Their results are consistent with those re-ported by Riikgauer et al.[242] (Table 3) in that no correlation was observed between serum manganese concentration and the gender of the subject and children had higher serum manganese concentrations when compared to adults (Tables 3 and 5). These investigators observed a mean (±SD) serum manganese concentration of 1.06 ± 0.62 μg manganese/L (range from 0.19 to 3.33 μg manganese/L). Whole blood manganese concentrations are sensitive to developmental life stage (Tables 3 and 5). Pregnant women and infants often have elevated blood manganese concentrations (Table 6).[245] Studies examining whole-blood concentrations of manganese during pregnancy show an increase throughout gestation (Table 6), and iron supplementation of pregnant women with normal iron status does not significantly change blood manganese concentrations.[246]

Several studies have shown that serum or plasma manganese concentrations respond to dietary intake. For example, Davis and Greger[208] showed that women consuming 1.7 mg manganese/ day had lower serum manganese concentrations than did women ingesting 15 mg manganese/day. A statistically significant positive correlation has been observed between serum manganese concentrations of human-milk-fed infants and both the manganese concentration of the consumed milk and the daily intake of manganese.[56]

TABLE 5
Manganese concentration in whole blood, plasma, and serum of males and females <18 yr of age (normal range 15–56 μg/L in whole blood; 1.4–15 μg/L in plasma)

Investigator	Country	Analysis method	Study description and number of subjects	Whole blood,[a] plasma,[b] or serum[c] manganese (μg Mn/L)
Spencer (1999)[245]	Australia	GFAAS	3 to 4-day-old neonates (22)	40.37 ± 11.55[a]*
Stastny et al. (1984)[56]	United States	GFAAS	3-mo-old infants (24)	4.7 ± 1.6[c]
Mizoguchi et al. (2001)[243]	Japan	AAS	Children	
			Neonates (14)	56.4 ± 16.4[a]
			1–11 mo (21)	26.1 ± 18.9[a]
			1–18 yr (36)	14.8 ± 3.8[a]
			1–18 yr with portosystemic shunt (7)	24 ± 4.3[a]
Acosta and Yannicelli (1999)[290]	United States	AAS	Infants	
			With phenylketonuria, 3 mo (19)	14.85 ± 3.9[b]**
			With phenylketonuria, 6 mo (23)	11.00 ± 1.1[b]**
Rükgauer et al. (1997)[242]	Germany	ETAAS	Children 1 mo–18 yr (129)	1.40 ± 063[c]*
			Children < 6 mo (13)	2.1 ± 0.81[c]*
			<1 yr (18)	2.1 ± 0.75[c]*
			<2 yr (15)	1.61 ± 0.84[c]*
			<4 yr (23)	1.36 ± 0.46[c]*
			<6 yr (19)	1.42 ± 0.62[c]*
			<10 yr (25)	1.24 ± 0.48[c]*
			<14 yr (21)	1.19 ± 0.39[c]*
			<18 yr (17)	0.96 ± 0.31[c]*
Alarcón et al. (1996)[291]	Venezuela		Infants 5 days (22)	0.45 ± 0.12[c]
			Infants 1 mo (20)	0.41 ± 0.11[c]
			Infants 3 mo (22)	0.39 ± 0.13[c]
			Infants 5 mo (14)	0.39 ± 0.1[c]
			Infants 7 mo (20)	0.38 ± 0.09[c]
			Infants 10 mo (20)	0.37 ± 0.11[c]
			Infants 11 mo (22)	0.36 ± 0.12[c]
			Infants 12 mo (40)	0.29 ± 0.1[c]

Note. Data are mean ± SD; *a*, *b*, *c* in final column refer to fluid in column heading. AAS, flame atomic absorption spectrophotometry; GFAAS, graphite furnace atomic absorption spectrophotometry; ETAAS, electrothermal atomic absorption spectrophotometry.
*Converted from nmol/L;
**Converted from μmol/L.

Freeland-Graves and Turnlund[247] showed that plasma manganese concentrations were reduced as human subjects consumed diets intended to deplete body stores of manganese. These investigators also showed that plasma manganese concentrations increased when the diets were supplemented with manganese. Serum and plasma manganese concentrations can be insensitive to large variations in dietary manganese intake and are not predictive of dietary intake. For example, Stastny et al.[56] showed that mean (±SD) serum manganese concentrations observed in infants fed human milk were identical to those seen in formula-fed infants (4.4 ± 1.8 vs. 4.7 ± 1.6 μg manganese/L) despite a >400-fold higher daily manganese intake in the formula-fed infants (0.42 vs. 183 μg manganese/kg).

Whole blood, serum, and plasma manganese concentrations have also been evaluated in individuals exposed to air-borne manganese, most commonly in an occupational setting (Table 4). The strength of the observed correlation between blood manganese concentrations and manganese exposure concentrations often depends on the magnitude and the duration of the exposure. In some studies, whole-blood manganese concentrations positively correlate with exposure to manganese (Table 4). In other cases, blood manganese concentrations in exposed workers remain in the normal adult range (4-14 μg manganese/L) and urine and hair manganese concentrations do not differ between exposed and non-exposed workers (Table 4). The poor correlation between air and blood manganese concentration may be related to the extremely short (~5 min) half-life of manganese in the blood following acute inhalation exposure.[248]

Lymphocytic Manganese Superoxide Dismutase Activity

Another potential biomarker of early biochemical effect of manganese (not necessarily exposure) is the determination of lymphocytic manganese-superoxide dismutase (MnSOD) activity. Davis and Greger[208]

demonstrated that lymphocytic MnSOD activity was elevated in 4 women supplemented with 15 mg manganese/day for more than 3 months. MnSOD is also an imperfect biomarker since it may also be affected by ethanol exposure,[249] nonheme iron,[86] strenuous exercise,[250] and diets rich in polyunsaturated fatty acids.[251]

TABLE 6
Manganese concentration in whole blood of pregnant women (normal range 4–14 μg/L in whole blood)

Investigator	Country	Analysis method	Study description and number of subjects	Whole blood manganese (μg Mn/L)
Spencer (1999)[245]	Australia	GFAAS	Pregnant females (34)	
			Trimester 1	8.25 ± 2.94*
			Trimester 2	9.46 ± 3.28*
			Trimester 3	12.65 ± 3.74*
Tholin et al. (1995)[246]	Sweden	GFAAS	Iron-supplemented vs. nonsupplemented pregnant women (74)	
			Iron-supplemented	
			Trimester 1	8.09*
			Trimester 2	11.28*
			Trimester 3	13.7*
			Iron nonsupplemented	
			Trimester 1	9.08*
			Trimester 2	10.73*
			Trimester 3	12.05*
Wilson et al. (1991)[292]	Ireland	ETAAS	Pregnant females (56)	
At 38 wk gestation				4.2 ± 1.9
Cord blood				5.4 ± 1.6

Note. Data are mean ± SD. GFAAS, graphite furnace atomic absorption spectrophotometry; ETAAS, electrothermal atomic absorption spectrophotometry.
*Converted from nmol/L.

Arginase Activity

Arginase activity is also sensitive to manganese tissue concentrations[252,253] and may hold some future promise as a biomarker of exposure for manganese.[13]

Magnetic Resonance Imaging (MRI)

Most tissue concentrations are relatively inaccessible in humans without the use of biopsy procedures and thus their usefulness as a biomarker is quite limited. One procedure that may hold a great deal of promise as a biomarker of exposure involves the use of magnetic resonance imaging (MRI) techniques. MRI is based on the absorption and emission of energy in the radio frequency range of the electromagnetic spectrum. Manganese is paramagnetic. Paramagnetic substances shorten the T1-weighted value obtained on MRI. MRI procedures have revealed that humans accumulate manganese within discrete brain structures including the striatum, globus pallidus, and substantia nigra.[5,46,77,254] These hyperintensities are bilateral, symmetrical, and visible in T 1-weighted magnetic resonance imaging of different manganese overload conditions. There have been numerous reports of clinical studies on relations or correlations among blood manganese concentrations, and intensity on T1-weighted images (MRI intensity).

For example, Takagi et al.[77] have shown a high degree of correlation between the T1-weighted intensity of the MRI image obtained from the globus pallidus and whole-blood manganese concentrations in human subjects *(n* = 12; 17 to 62 years of age at the time of entry on the study) that were given total parenteral solutions providing either 0, I , 2, or 20 μmol manganese/day for 4.6 to 19.5 months. Both manganese blood concentration and Pallidal Index (PI)* represent highly variable measurements (the former not representing a good indicator of chronic manganese exposure) and with time may be developed as an accurate biomarker of manganese exposure.[255] However, although patients with different T1-weighted intensities are recognized clinically, there

have been few efforts made to determine whether image intensity directly correlates with brain manganese concentrations.

Gallez and coworkers[256] recently reported a positive correlation between NMR proton T1 relaxation times (obtained using a 4.7 Tesla imager) and brain manganese concentrations as determined by atomic absorption spectrometry in manganese-exposed rats. It is likely that a similar approach could also be applied to human clinical imaging studies; however; there is in-sufficient evidence to suggest that data from Gallez et al.[256] could be used directly to estimate brain manganese concentrations in exposed humans.

ADDITIONAL FACTORS THAT CAN INFLUENCE MANGANESE TISSUE CONCENTRATIONS

Manganese Deficiency

Dietary intake of manganese is known to influence the amount of manganese absorbed from the gastrointestinal tract and the amount of manganese eliminated in the bile. When dietary manganese levels are high, adaptive changes often include reduced gastrointestinal absorption of manganese, enhanced manganese liver metabolism, and increased biliary and pancreatic excretion of manganese.[18,86,92-97]

Few investigators have examined whether dietary manganese levels can influence the pharmacokinetics of inhaled manganese. Moore and coworkers[257] evaluated tissue manganese concentrations in rats fed either a 5- or 42.2-ppm manganese diet and exposed subchronically (8 h/day for 56 consecutive days) to irradiated exhaust from an automobile engine using gasoline containing MMT. These authors reported that brain, kidney, and liver manganese concentrations were lower in air-exposed control animals fed a 5-ppm manganese diet compared to animals fed the 42.2-ppm manganese diet. Animals fed either the 5- or 42.2-ppm manganese diet and exposed to irradiated exhaust (containing approximately 117 μg Mn/m^3) developed a 45 or 84% increase in total brain manganese concentrations, respectively, compared with air-exposed controls (i.e., no manganese in air). However, the results of this study were not analyzed in such a manner as to determine whether diet and inhalation inter-active effects occurred. Although not determined directly during this study, the most likely form of manganese in the engine exhaust was the tetroxide, Mn304, since a catalytic converter was not used in the generation system.[258]

Recent studies by Dorman et al.[93,94] determined the influence of manganese body burden on the pharmacokinetics of inhaled manganese sulfate ($MnSO_4$) and manganese tetraoxide (Mn_3O_4) in neonatal rats (postnatal day 10; PND 10) reared on either a low (2 ppm), sufficient (10 ppm), or high (100 ppm) manganese diet. Once tissue manganese concentrations stabilized (i.e., after 2 months on the special diets), male rats were exposed by whole-body inhalation for 6 h/day on 14 consecutive days to $MnSO_4$ or Mn_3O_4 at concentrations equivalent to 0, 0.03, or 0.3 mg manganese/m3. Dorman and coworkers then evaluated a variety of tissues for their manganese content and determined the rate of manganese elimination by whole-body gamma spectrometry following intravenous administration of a ^{54}Mn tracer.

As expected, Dorman and coworkers[93,94] found that the feeding of a marginally deficient diet containing only 2 ppm manganese was associated with a number of effects, including reduced body weight gain, decreased liver manganese concentrations, and reduced whole-body manganese clearance rates. They also showed that biliary excretion of manganese was influenced by oral manganese intake. Rats kept on the marginally manganese-deficient diet had decreased biliary manganese concentrations and slower elimination of the ^{54}Mn tracer when compared with rats given manganese-sufficient diets. Male rats exposed to 0.3 mg $manganese/m^3$ developed increased manganese concentrations in some tissues; however, this study did not demonstrate significant diet and inhalation interactions on brain manganese concentrations. Despite being in a relatively manganese-deficient state, rats given the 2-ppm manganese diet and exposed to $MnSO_4$ or Mn_3O_4 increased their rate of manganese excretion when compared to their respective air-exposed control groups. This observation suggests that, at least initially, under these experimental conditions animals will not retain inhaled manganese to compensate for relative nutritional deficiencies in this essential metal.

Lifestage

The pharmacokinetics of manganese during critical phases of development has been most thoroughly examined using the oral route of exposure. As with other xenobiotics, manganese exposure to the developing nervous system is a function of the dose administered to the dam and the pharmacokinetics of the com-pound in the maternal, placental, and embryo/fetal (or neonatal) circulations.

Concern exists that elderly individuals may be another sensitive subpopulation at increased risk for the onset of neurologic disorders and that elevated exposures to manganese in the pre-parkinsonism state might be associated with an increased incidence of Parkinson's disease. In a study where a pre-parkinsonian state was induced by a unilateral intrastriatal injection of 6-hydroxydopamine (6-OHDA, a dopaminergic neurotoxin), followed 4 weeks later by exposure to manganese (4.8 mg manganese/kg x 3 intraperitoneal injections/week, for 5 weeks), rat brain manganese levels were 3.4-fold increased in over controls.[259] Notably, manganese exposure in the presence of a pre-parkinsonian state (but not in its absence) also significantly exacerbated some neurobehavioral effects of manganese suggesting that manganese exposure may increase the risk of neurological impairment in elderly populations with a pre-parkinsonian state.

Dorman et al.[260] recently completed a series of experiments to evaluate whether manganese pharmacokinetics is altered in aged animals. Dorman et al.[260] determined the pharmacokinetics of manganese phosphate and manganese sulfate ($MnSO_4$) in young adult and senescent (> 16-month old) male CD rats following subchronic inhalation exposure (6 h/day, 5 days/week, for 13 weeks). Nominal $MnSO_4$ exposure concentrations of 0, 0.01, 0.1, and 0.5 mg manganese/m^3 and a single manganese phosphate exposure concentration of 0.1 mg manganese/m^3 were used. Rats were evaluated at the end of the 90-day exposure and at 45 days postexposure. An overall age-related main treatment effect was observed on some tissue manganese concentrations. Young male rats had significantly higher olfactory bulb, blood, femur, and pancreas manganese concentrations when compared to senescent male rats. Increased olfactory bulb manganese concentrations in young rats are most likely related to a portal-of-entry effect arising from a higher pulmonary ventilation rate in young animals. Young male rats also had significantly lower testes manganese concentrations when compared to old males. The aging rat testes have been shown to accumulate other metals. One possible explanation for increased manganese delivery to the testes of senescent animals is decreased integrity of the seminiferous epithelium and the blood—testis barrier that occurs in aged rats.[261] The results of this study do not suggest that the aged rat nervous system accumulates more manganese than in young animals.

Iron Deficiency

Worldwide, the prevalence of iron deficiency anemia (IDA) in infants and children is estimated to be at approximately 25%.[262] A report from the World Health Organization estimates that 46% of the world's 5- to 14-year-old children and 48% of the world's pregnant women are anemic.[263,264] A majority of these cases of anemia are due to iron deficiency. According to data from the third National Health and Nutrition Examination Survey (NHANES III, I 988-1994),[265] the prevalence of IDA in U.S. children between 1 and 2 years of age is 3% and the prevalence of iron deficiency without anemia is 9%. This prevalence corresponds to ~240,000 and 700,000 infants, respectively. Further-more, the prevalence of iron deficiency among women of child-bearing age (20-49 years of age) has been reported to range from 8 to 20%,[266] suggesting that infants may be at risk for marginal to low iron status not only as a result of postnatal factors, but also as a result of limited prenatal iron sources.

The neurobiological consequences of iron deficiency include alterations in behavior, cognition, and neurotransmitter metabolism.[176,267,268] Offspring of marginal iron dams demonstrate lower grip strength, attenuated startle responsiveness, and altered performance in the Morris water maze. These differences in performance were found in association with lower brain iron concentrations. Notably, iron deficiency is also associated with decreased dopamine D2 receptors in striatum,[269,270] increased extracellular dopamine concentrations,[271-273] decreased dopamine transporter and dopamine receptor functioning,[274,275] thus sharing a number of commonalities with manganese-induced neurotoxicity.[276] These commonalities raise the possibility that the neurological seguelae of iron deficiency are mediated, or, at least potentiated, by increased CNS

manganese concentrations. Additional research will be required to determine whether this interaction indeed occurs.

DMT-1 mRNA levels are significantly increased in patients with iron deficiency and hereditary hemochromatosis, whereas they are unchanged in patients with secondary iron overload.[277] Alterations in DMT-1 mRNA levels are paralleled by comparable changes in the duodenal expression of these proteins. In patients with normal iron status or iron deficiency, significant negative correlations between DMT-1 mRNA and serum iron level parameters have been noted. Manganese levels in these patients have not been determined, and no data are currently available on the potential that increased DMT-1 mRNA expression translates to increased plasma or brain manganese load. Maternal iron deficiency during pregnancy induces anemia in the developing fetus; however, the severity tends to be less than in the mother as a result of an increased efficiency of iron flux.[132]

In a recent study[176] weanling rats were fed one of three semipurified diets: control, iron deficient (ID), or iron deficient/manganese fortified (IDMn+). Plasma transferrin (significant increase) and iron concentrations (significant decrease) were characteristic of severe anemia in the ID and IDMn+ groups.[176] Seven brain regions (caudate putamen, globus pallidus, thalamus, hippocampus, substantia nigra, cerebellum, and cortex) were analyzed for manganese concentration. Both ID and IDMn+ diets caused significant increases in manganese concentration across brain regions compared to control diets; however, increased dietary manganese (IDMn+) did not increase brain manganese concentration beyond the level associated with ID, except in the hippocampus.

Vegetarian Diets

Increasing numbers of young people are adopting a vegetarian lifestyle. The effect of vegetarian diets on iron status is controversial. In a recent study, Pongstaporn et al.[278] reported on hematological parameters and serum ferritin in 179 vegetarians and 58 control (with no known disease) subjects. It was found that hemoglobin, hematocrit, and serum ferritin in vegetarians were significantly lower than in control subjects. There were 34 cases of iron-deficiency in 179 vegetarians (19%), which can be classified to iron depletion (4 cases), iron-deficient erythropoiesis (12 cases) and iron-deficiency anemia (18 cases). Conversely, Hunt et al.[279] suggested that iron status in women consuming controlled lacto-ovovegetarian diets for 8 weeks is unaltered compared to women on a nonvegetarian diet. The physiologic adaptation was reflected by an increase in the efficiency of iron absorption and insensitivity of blood iron indexes, including serum ferritin, despite 70% lower nonheme iron absorption from a lacto-ovovegetarian diet. At the time of this writing, the relationship between vegetarian lifestyle, adaptation to increased iron absorption (potentially via the up regulation of DMT-1), and manganese uptake from a diet likely to exceed normal manganese intake (given the higher content of manganese in vegetarian food of plant origin) is undetermined. Thus the combination of iron deficiency and a diet skewed to higher than usual manganese levels (vegetables, cereals, etc.) in vegetarians should be considered for its potential adverse interaction (i.e., inverse diet-manganese interaction).

CONCLUSIONS

Much is known about the dosimetry of manganese; however, this information has been incompletely integrated into rigorous quantitative risk assessments for this metal. Future risk assessments would benefit from the development of physiologically based pharmacokinetic (PBPK) models for manganese. The power of biologically based dosimetry models rests in their ability to estimate the amount of the active form of a chemical at its target site within the body over time, given virtually any exposure paradigm. Risk assessments will need to consider life stage since this is an important determinant of manganese pharmacokinetics and response. Comprehensive risk assessments for manganese should also consider oxidation states of manganese in the blood, uptake rates of protein-bound forms of manganese by the liver, neuronal transfer rates within the brain, olfactory transport to brain manganese delivery, and mechanisms of manganese-induced neurotoxicity.[12,166]

Note:
* PI represents the ratio of globus pallidus to subcortical frontal white-matter signal intensity in T1-weighted MRI planes multiplied by 100.

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